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**MEASURING THE TEMPERATURE RESPONSE OF
SOIL RESPIRATION FROM TWO DISTINCT
CARBON POOLS IN SOIL**

A thesis
submitted in fulfilment
of the requirements for the degree
of
Doctor of Philosophy in Earth Science
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JASMINE MAY ROBINSON



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Abstract

As soils contain a large store of terrestrial carbon, understanding the dynamics and stability of this important carbon reserve and how it may change with regards to future changes in temperature is of global interest. It is primarily understood that carbon exists in different pools within soil, but there is considerable debate around the number, size and contents of these pools. Despite this debate, when modelling carbon cycling, it can be erroneously assumed that the decomposition of carbon pools will behave the in the same way with regards to temperature and varying management practices.

The first objective of this research was to develop and test a new protocol allowing the measurement of the temperature response of two distinct carbon pools in soil. A Horotiu silt loam was mixed with a ^{13}C labelled rye-grass clover litter, and incubated for 5 and 20 hours at 30 discrete temperatures ($\sim 2 - 50^\circ\text{C}$). Resulting CO_2 was separated into litter and soil organic carbon (SOC) sourced respiration rates using a mixing model, and then fit with macromolecular rate theory (MMRT). Litter-derived respiration had a lower temperature optimum (T_{opt}) than SOC-derived respiration. It was suggested that decomposition of highly available labile litter is rate-limited by enzyme kinetics, which displays a clear temperature optimum. In contrast, decomposition of stable SOC is more limited by desorption processes and diffusion of carbon to microbes, prior to decomposition, and so exhibits Arrhenius behaviour as temperature increases.

The second objective was to use the developed protocol to measure temperature response of respiration was measured for ^{13}C -labelled new photosynthate carbon inputs and bulk SOC, partitioned from soils labelled under seasonal irrigated and dryland conditions. Additionally, mass litter inputs of both root and shoot material were incubated with unlabelled soil. Root and shoot litter inputs showed a similar response to temperature with a well-defined MMRT-like response (T_{opt} of 45 °C and 38 °C respectively). In contrast to this, respiration from new photosynthate carbon and SOC had the same Arrhenius-like temperature response (T_{opt} of 50 °C for dryland and 62 °C for irrigated soils). It was suggested that the new carbon inputs deposited through roots were rapidly incorporated into the soil and thus had a similar availability and temperature response as SOC. Consequently, carbon inputs through roots appear to be more stabilised than litter inputs (as either root or above ground fragments). Respiration from dryland soils had lower a T_{opt} than respiration from irrigated soils, which diminished with the application of autumnal rainfall, most likely due to an increase in short-term turnover of carbon under irrigation.

In a similar experiment, soils were labelled under constant conditions prior to the imposition of seasonal irrigation or dryland treatments. Again, there was no difference between the temperature response of new photosynthate carbon and SOC. Increases in short term carbon cycling caused greater respiration under irrigation compared to dryland soils. However, this increased respiration did not contribute to a noticeable change in temperature response.

Overall this research demonstrated a reliable protocol for measuring the temperature response of two distinct carbon pools in soil. This approach can be used to examine

the stability of new carbon inputs from different sources to soil. Future research using the developed methodology with different forms of ^{13}C labelled carbon will expand knowledge on the temperature response of distinctive pools of carbon in soil, allowing continued improvement on carbon cycling modelling.

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Chapter 1: Introduction

1.1 Carbon Cycling

The global carbon cycle is one of the most important biogeochemical cycles as it not only impacts food production and soil stability, but also contains the processes involved in the cycling of carbon dioxide (CO₂), a key controller of the Earth's climate (Lehmann & Kleber, 2015; Rousk & Bengtson, 2014). The largest actively cycling reserve of carbon is that of soil organic carbon, the storage of which is fundamentally based on the balance of plant photosynthetic inputs and microbial decomposition, often measured as respiration (Friedlingstein et al., 2019; Janzen, 2004; Schlesinger & Andrews, 2000). Microbial decomposition is very sensitive to small changes in environmental factors such as temperature, moisture content, and pH that control microbial growth potential or availability of carbon supply for consumption (Conant et al., 2011; Davidson & Janssens, 2006). Small changes to these factors can cause substantial increases to CO₂ outputs and declines in total carbon storage, thus understanding how future temperature changes and anthropogenic activities such as agriculture may impact microbial decomposition and carbon storage is crucial for managing both future food security and the changing climate (Brusseau, 2019; Rousk & Bengtson, 2014).

Microbial respiration is temperature dependant in two ways; through indirect effects of temperature on substrate availability, and direct effects on microbial metabolism (Conant et al., 2011). The observed temperature response of microbial respiration is the measured result of these processes at a specific point in time and space. A large

body of research exists that analyses the temperature response of microbial respiration over a multitude of distinctive ecosystems and soil types, under differing management practices, and over various temporal ranges. However, confusing terminology, inconsistent methodologies, and model choices hinder the establishment of a community consensus on the general effect temperature seems to have on microbial respiration (Conant et al., 2011; Luo et al., 2016; Wieder et al., 2015).

Commonly used models such as Arrhenius or Lloyd and Taylor equations typically fit limited data sets sufficiently (Alster et al., 2020; Robinson et al., 2017; Schipper et al., 2014). However, these models are exponential in nature and do not account for the decline in respiration rate past a temperature optimum, which is often seen in biological rates (Alster et al., 2020; Schipper et al., 2014). Macromolecular rate theory (MMRT) is based on the hypothesis of a changing heat capacity during enzyme catalysis with increasing temperature and captures the decline in respiration rate while provides a theoretical background for model fitting (Alster et al., 2020; Schipper et al., 2014). This model predicts additional, potentially useful parameters, such as T_{opt} (the temperature at which respiration is maximal) and T_{inf} (the point at which the change in respiration rate is greatest), for comparisons between analyses (Alster et al., 2020; Schipper et al., 2014).

1.2 Temperature response of different carbon pools in soil

It is mostly accepted that carbon storage in soil is not consistent throughout the soil matrix and is controlled by a multitude of differing biological, physical and chemical processes, all of which can theoretically determine its response to environmental and

anthropogenic influences (Conant et al., 2011; Davidson & Janssens, 2006). Defining and reliably separating carbon into functional pools that can reveal potential relationships between carbon allocation, stabilisation mechanisms, transfers, and sensitivity to environmental changes is key for improving the understanding of global carbon cycling (Conant et al., 2011; Luo et al., 2016; Wieder et al., 2015).

There are many methodologies used to measure the size of functional carbon pools in soil. Physical fractionation methods such as aggregate size fractionation, particle size fractionation or density fractionation aim to separate carbon pools based on physical protection (Dungait et al., 2012; Jackson et al., 2017; Poeplau et al., 2018). On the other hand, chemical fractionation methods like solubility extractions, hydrolysis, oxidation techniques separate carbon pools based on inherent chemical quality or aim to mimic *in situ* degradation (Helfrich et al., 2007; Poeplau et al., 2018). In addition to these methods, the isotopic tracers ^{13}C and ^{14}C can be used to identify individual carbon pools without direct interference or disruption of the soil matrix (Zacháry, 2019). Isotopic procedures can identify at least two pools of carbon using either natural shifts in ^{13}C value, such as those associated with changes in vegetation from C3 to C4 species or by using artificially labelled carbon, added to soil through plant uptake of enriched CO_2 (Trumbore & Zheng, 1996; Zacháry, 2019).

Despite multiple methods of separating pools, in general, it is assumed there are at least two pools of carbon in soil, an actively cycling, labile pool that is readily consumable, and a stored pool that is protected from decomposition (Dungait et al., 2012; Lehmann & Kleber, 2015; Schmidt et al., 2011). The temperature sensitivities for these two generalised pools are often compared in the literature. As stated by

multiple large syntheses (Conant et al., 2011; von Lützow & Kögel-Knabner, 2009; Wang et al., 2019), the most common finding was that stable carbon had an overall higher temperature sensitivity (Q_{10}) when compared to the corresponding labile carbon pool. However, the existence of contrasting conclusions, large variations in Q_{10} , and a lack of consistency regarding the methods of carbon pool fractionation, soil incubation techniques and temperature response models being used, increases the uncertainty attached to this generalisation (Conant et al., 2011; von Lützow & Kögel-Knabner, 2009; Wang et al., 2019). Conant et al. (2011) suggested that the temperature sensitivity of soil respiration was likely controlled by three temperature dependant processes: the depolymerisation of carbon (the degradation of molecules into smaller decomposable fractions); the rate of enzyme production, and substrate availability. In order to understand the temperature dependence of multiple carbon pools in soil, studies that could improve the understanding of temperature sensitivity of soil respiration should aim to separate the response of these factors and use comparable methodologies (Conant et al., 2011).

1.3 Impacts of seasonal irrigation

While understanding physical drivers of carbon cycling, like temperature, is important, the impacts of these drivers are also modified by land use and management. As up to 70% of agricultural land is managed grassland, management strategies such as irrigation (which can alter the balance of inputs and outputs of carbon under these systems) may significantly impact global carbon storage (Mudge et al., 2017; Whitehead et al., 2018).

Typically, irrigation can increase above-ground biomass, and in some cases, also reduce root biomass and increase microbial decomposition of soil carbon (Scott et al., 2012; Trost et al., 2013). Often studies have found these changes seem to result in a loss of total carbon under irrigation (Condrón et al., 2014; Kelliher et al., 2012; Mudge et al., 2017; Schipper et al., 2013). However, despite dependence on summer irrigation, New Zealand based studies on carbon storage under irrigation are limited and overall inconclusive (Whitehead et al., 2018). Furthermore, most irrigation studies focus on changes to total carbon, which may provide an understanding of total carbon stocks, but do not consider irrigation effects on the allocation and transfer of carbon between labile and stabilised carbon pools within a soil profile. Ultimately to understand and improve carbon storage under these essential ecosystems, further detail of the dynamics of soil carbon cycling is needed (Jackson et al., 2017).

To compare the mechanisms of carbon allocation and storage under typical New Zealand dryland or seasonal irrigation, Carmona et al. (2020) used enriched CO₂, (¹³CO₂) pulse labelling to trace carbon allocation and storage of a ryegrass/clover pasture and carbon cycling under these conditions. They found that irrigation did not increase total carbon allocation over the irrigation period, nor did it reduce the soils total carbon content (Carmona et al., 2020). However, when soil was fractionated into size fragments, irrigation did lead to differing amounts of carbon in the fine particulate organic matter (POM) fraction (53–250 µm) and the clay fraction (< 5 µm) compared to dryland treatments (Carmona et al., 2020). It was suggested that changes to new material in the fine POM clay fractions might be caused by potential stabilisation through increased root turnover, aggregation, and of carbon under irrigated pasture due to increased biological activity under irrigated treatments (Carmona et al., 2020).

While the mentioned studies often attempt to determine changes to inputs or storage of carbon within soil, losses through microbial decomposition and its response to temperature are of equal importance. Schipper et al. (2019) used the methodology described in Robinson et al. (2017) to examine the temperature response of soil respiration from 32 paired, irrigated and non-irrigated sites throughout New Zealand. They found that on average, irrigated sites had lower respiration rates and higher T_{opt} and T_{inf} values, which were attributed to lower carbon availability for microbial decomposition (Schipper et al., 2019). Furthermore, they also found irrigated soils had a higher temperature sensitivity (Q_{10}), which was also linked to lower carbon availability as less labile substrates are usually linked to higher temperature sensitivities (Conant et al., 2011; Larionova et al., 2007; von Lützow & Kögel-Knabner, 2009).

Differences in temperature response, as well as a potentially lower total carbon content under irrigation (Condon et al., 2014; Kelliher et al., 2012; Mudge et al., 2017; Schipper et al., 2013), highlights the need for detailed analysis of carbon cycling and stability under irrigation to understand the potential long term impacts of extensive and critical management practice (Jackson et al., 2017). Additionally, there is a large stable pool of carbon in soils that is highly temperature sensitive, a lack of consistency in model choice could underestimate the degree to which this pool will decompose under accelerated climate warming (Bradford et al., 2016; Zhou et al., 2018).

1.4 Thesis aim and objectives

The main aim of this thesis was to use ^{13}C enrichment methodologies to separate the temperature dependence of respiration from two distinct carbon pools in soil. This

research was carried out to address the inconsistencies in current methodologies and to increase the understanding of temperature and management effects on respiration from differing carbon pools in soil.

Specific research objectives:

- 1) To establish a new protocol for measuring the temperature dependence of respiration from two distinct carbon pools in soil, using ^{13}C enrichment methodologies,
- 2) To compare the temperature response of respiration from new inputs of photosynthate carbon and soil organic carbon and determine whether the deposition of these new inputs under either irrigated or dryland conditions alter the temperature response,
- 3) To compare the temperature response of respiration from new inputs of photosynthate carbon and soil organic carbon and determine whether the subsequent application of seasonal irrigation alters the temperature response of these two carbon pools.

General hypotheses to the above research objectives were:

- 1) Labelled litter additions to soil will have a lower T_{opt} and T_{inf} than soil organic carbon and higher temperature sensitivity (Q_{10}) due to the relative carbon availability of mass litter inputs for microbial decomposition,
- 2) A reduction in available carbon under irrigation due to increased carbon cycling will result in a higher temperature response (T_{opt} and T_{inf}) and a lower

temperature sensitivity (Q_{10}). New photosynthate carbon may have a lower temperature response due to relatively higher carbon availability,

- 3) Increased carbon cycling during seasonal irrigation application will reduce the carbon availability and result in a higher temperature response and sensitivity (T_{opt} , T_{inf} and Q_{10}) of respiration under these conditions.

1.5 Thesis structure

The structure of this thesis following this introduction:

- Chapter 2 is a literature review with an in-depth analysis of the effects of temperature on different aspects of microbial decomposition. This review provides a background of the temperature dependence of soil respiration, identifying confusing terminology and methodologies while identifying knowledge gaps like the effects of seasonal irrigation on carbon cycling,
- Chapters 3, 4 and 5 are experimental work presented in manuscript format. They cover the specific objects outlined above. Briefly, Chapter 3 describes a new protocol for measuring the temperature dependence of two distinct carbon pools in soil. Chapters 4 and 5 present temperature response results using the newly developed protocol with both soils labelled under seasonal irrigated and dryland conditions, and soils labelled under constant conditions, followed by the application of seasonal irrigation. As these chapters are presented as papers, there is some repetition between chapters, particularly in the methodologies. As of the time of submission, Chapter 3 (Robinson et al., 2020) has been published in *Biogeochemistry* (150 (1), 45-59). Chapters 4 and 5 are yet to be submitted to a peer-reviewed journal,

- Chapter 6 summarises the main results and conclusions of the previous chapters and provides some broader implications of the research, including identifying of areas for future research.

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Chapter 2. Literature Review

2.1 Introduction

Biogeochemical cycles include the movement, transformation and storage of the most common chemical elements between the biosphere, atmosphere, lithosphere and hydrosphere (Brusseau, 2019). As these cycles relate to most natural processes, understanding the mechanisms and current state of elemental stocks is crucial to understanding the functioning of the world around us (Brusseau, 2019; Rousk & Bengtson, 2014). One of the most important biogeochemical cycles is the global carbon cycle, which in addition to directly impacting food production and soil stability, also contains the cycling of carbon dioxide (CO₂), one of the key controllers of the Earth's climate (Lehmann & Kleber, 2015; Rousk & Bengtson, 2014). As anthropogenic activities, such as agriculture, may affect multiple parts of the carbon cycle, understanding how this cycle operates and may change in the future is essential for managing our changing climate and future food security (Brusseau, 2019; Rousk & Bengtson, 2014).

2.1.1 Global carbon cycle

Three main reservoirs of carbon exist in the global carbon cycle, and the movement between these pools is driven by a combination of chemical, physical and biological processes (Figure 2.1; Schlesinger & Andrews, 2000). These three pools are atmospheric carbon (e.g. CO₂ and CH₄), terrestrial carbon (mostly in soil and vegetation), and oceanic carbon (Janzen, 2004).

The global carbon cycle

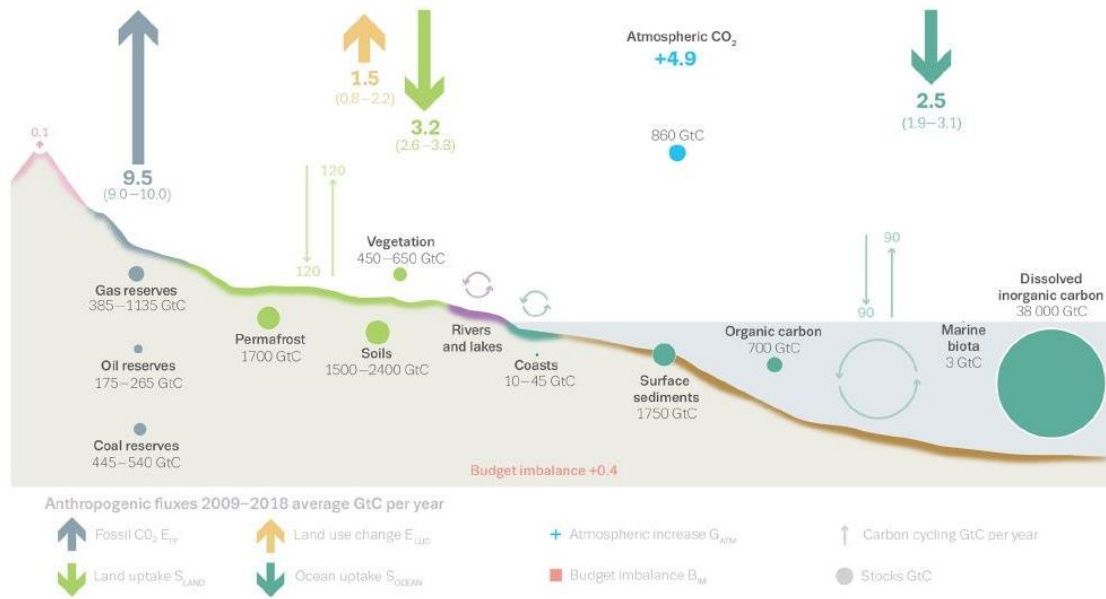


Figure 2.1: Synthesis of current knowledge of the global carbon cycle and carbon stocks as presented in Global Carbon Budget, 2019 (Friedlingstein et al., 2019). From Friedlingstein et al. (2019): “Schematic representation of the overall perturbation of the global carbon cycle caused by anthropogenic activities, averaged globally for the decade 2009–2018. See legends for the corresponding arrows and units. The uncertainty in the atmospheric CO₂ growth rate is very small (± 0.02 GtCyr⁻¹) and is neglected for the figure. The anthropogenic perturbation occurs on top of an active carbon cycle, with fluxes and stocks represented in the background and taken from Ciais et al. (2013) for all numbers, with the ocean gross fluxes updated to 90 GtCyr⁻¹ to account for the increase in atmospheric CO₂ since publication, and except for the carbon stocks in coasts which are from a literature review of coastal marine sediments (Price and Warren, 2016)” (p. 1783).

The largest reserve in this cycle, by a considerable margin, is dissolved inorganic carbon in the ocean. However, only a small percentage of this is considered active for cycling (~ 1000 GtC), while the rest is stored in the deep ocean (Friedlingstein et al., 2019; Janzen, 2004; Schlesinger & Andrews, 2000). The next largest pool, and the largest actively cycling pool, is soil, which exists mostly as organic carbon and lies within the first metre of soil (Figure 2.1; Janzen, 2004; Lal, 2004; Schlesinger & Andrews, 2000).

Storage of soil organic carbon is based on a balance of carbon inputs and outputs. Plant photosynthesis is the main pathway for the fixation of atmospheric carbon into soil. Photosynthetic inputs include above-ground litter decomposition and leachates, below-ground litter and root decomposition, and root/mycorrhizae fungi exudates (Gougoulas et al., 2014). Outputs are dominated by plant respiration and decomposition/respiration by soil microfauna, macrofauna and most importantly, microbes (Davidson & Janssens, 2006). Microbial respiration is very sensitive to changes in environmental factors, such as moisture or temperature, and small changes in variables can have a major influence on the amount of CO₂ released into the atmosphere (Conant et al., 2011; Fang & Moncrieff, 2001; Schlesinger & Andrews, 2000).

The balance and storage of soil carbon can also be affected by anthropogenic activities, like agriculture, which contributes significantly to yearly greenhouse gas emissions (Lal, 2004). Managed grasslands occupy ~70% of agricultural land and store a significant quantity of carbon in the soil (Whitehead et al., 2018). Consequently, small changes to the management of these grasslands may have significant effects on global carbon stocks (Whitehead et al., 2018). Studies have investigated how different management strategies, such as irrigation, affect carbon inputs and storage under grassland systems (Mudge et al., 2017; Whitehead et al., 2018). However, how these changes may affect carbon outputs, such as microbial respiration, and how this may respond to temperature increases is less known. As these grassland areas are important carbon stores and areas for potential carbon sequestration (Lal, 2004), furthering the knowledge of microbial decomposition under varying management practices is required.

2.1.2 Purpose and structure of literature review

The purpose of this literature review is to overview the complex biochemistry of soil microbial respiration and its controls and highlight the need for a new protocol for measuring the temperature response of soil respiration. Firstly, I will discuss microbial respiration and its controls such as pH, moisture content, substrate availability with a particular emphasis on temperature controls. I will review commonly used models of temperature response and calculations of temperature sensitivity. Lastly, I will consider different methods of identifying and fractionating carbon pools, followed by a brief discussion on the current knowledge on the temperature response of distinct carbon pools in soil. Carbon inputs through photosynthesis is also an important factor in carbon stability but is not the focus of this thesis.

2.2 Microbial respiration and its control

One of the main transfers of carbon back into the atmosphere is through heterotrophic microbial respiration, which is respiration derived from the microbial decomposition of soil organic carbon (Gougoulas et al., 2014; Janzen, 2004; Xu & Shang, 2016). Microbial respiration can be controlled by primary factors such as substrate availability (Section 2.3), temperature (Section 2.4), moisture (Section 2.2.1), and pH (Section 2.2.3), all of which influence either microbial growth potential or the availability of carbon supply to microbes for consumption (Xu & Shang, 2016). These primary factors can vary significantly both spatially and temporally, being influenced by many of external variables, including climate, soil type, plant species, land-use change, or irrigation and tillage (Wiesmeier et al., 2019; Xu & Shang, 2016). As these external variables are continually changing by anthropogenic or natural means, it is crucial to

understand how microbial respiration responds to these primary controls in order to improve future predictions of global soil carbon stocks (Janzen, 2004; Wiesmeier et al., 2019; Xu & Shang, 2016).

2.2.1 Substrate availability

Not all soil organic carbon in soil is similarly available to microbes for decomposition, it is often protected or ‘stored’ within the soil matrix (Dungait et al., 2012; Lützow et al., 2006). Three common factors that have been suggested to restrict substrate availability for microbial consumption in soil are: the occlusion of carbon in aggregates, the adsorption of carbon onto organic and mineral surfaces, and the chemical structure of the carbon compound itself (Dungait et al., 2012; Lützow et al., 2006). These will be discussed in greater detail in Section 2.3.

2.2.2 Moisture

Soil moisture affects microbial respiration in two major ways: i) the transport of gases to microbial sites and ii) the transport of solutes to microbial sites (Manzoni et al., 2012; Moyano et al., 2013). In a more minor capacity, soil moisture can also alter microbial metabolic costs and predator activity (Moyano et al., 2013).

In order for soluble carbon to be available for microbial decomposition, there needs to be sufficient water for the diffusion and transport of carbon within the soil matrix sites (Manzoni et al., 2012; Moyano et al., 2013). As soil moisture declines, the number of soil/water boundaries in the soil pore space, where diffusion can occur, also decline. This reduction of diffusion restricts new substrates from entering the water and thus

interacting with soil microbes. Additionally, the connectivity between soil aggregates is restricted, and the transportation of any new substrate to sites of microbial activity is also reduced (Manzoni et al., 2012; Moyano et al., 2013). These limitations lead to a drop in microbial decomposition/respiration rates. Furthermore, with restrictions on water availability, microbes begin to display physiological stress and adapt to conserve their cellular water content, increasing the metabolic cost of survival and decreasing the efficiency of microbial decomposition (Moyano et al., 2013).

Conversely to the above relationships, increasing soil moisture can inhibit oxygen diffusion and increase the predation of microbes. As with substrate diffusion, water is needed for the diffusion of oxygen to microbes in order for aerobic decomposition to occur (Manzoni et al., 2012; Moyano et al., 2013). However, as soil moisture content increases, water can displace air pockets within soil pores, reducing the amount of air/water boundaries where this diffusion of oxygen occurs. In these cases, anaerobic decomposition takes over, a generally less efficient method of carbon consumption for most soil microbes (Manzoni et al., 2012; Moyano et al., 2013). Also, as water content increases, predatory microfauna gain greater mobility reducing microbial populations and hence decomposition rates (Moyano et al., 2013).

Due to the opposing nature of these relationships to water content, microbes tend to have an optimum moisture content at which they function at maximum capacity. As summarised by Moyano et al. (2013), the ideal soil moisture content for microbial activity is a result of a balance between the four factors of; i) gas transport; ii) solute transport; iii) metabolic costs, and iv) predation (Figure 2.2; Moyano et al., 2013).

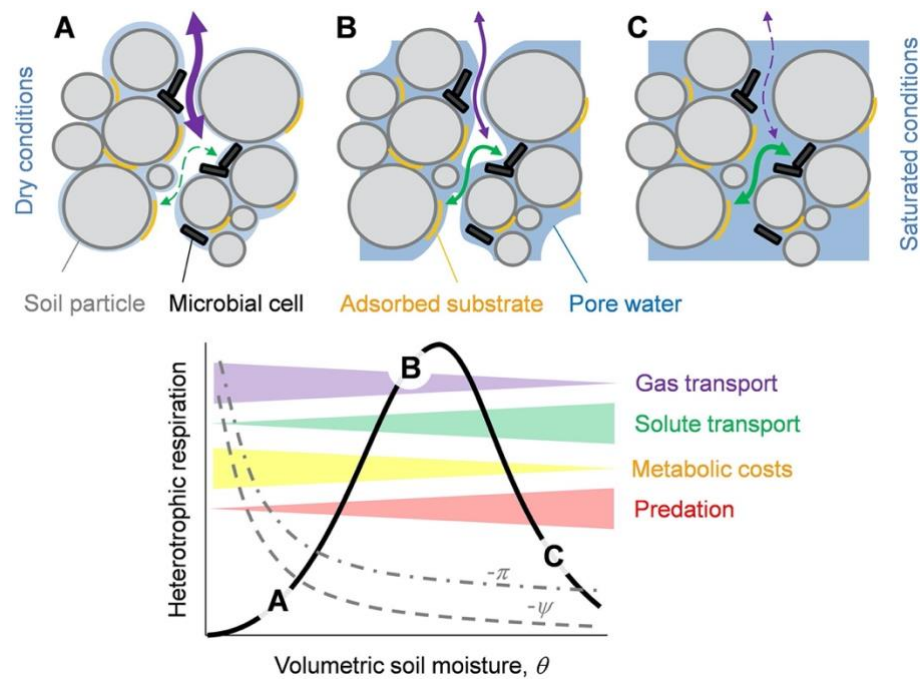


Figure 2.2: From Moyano et al. (2013): “Schematic illustration of soil moisture effects on microbial activity. The relationship between heterotrophic respiration and water availability in soils is the macroscopic result of a number of interacting effects, ranging from diffusion limitations to physiological, biochemical, and ecological processes. Because these effects often act in different directions (e.g., substrate transport decreases with decreasing soil moisture, whereas oxygen transport increases), a peak in respiration occurs at intermediate values of soil moisture. In the lower panel, $-\psi$ indicates the soil water potential and $-\pi$ is the cell osmotic potential that would allow maintaining a stable turgor pressure as ψ declines” (p. 74).

Despite a general understanding of the relationship of microbial respiration to soil moisture content as described above, in reality, the relationship is more complex (Yan et al., 2016). Soil properties that influence soil moisture content, including soil structure, texture, and organic matter content, are often not uniform within a soil matrix and can differ on a site by site basis within the soil matrix, which can ultimately vary microbial respiration (Yan et al., 2016).

2.2.3 pH

pH is a measure of the concentration of free hydrogen ions (H^+) in solution. A high pH solution (basic) will have a low concentration of H^+ ions, whilst a low pH solution (acidic) will have a high concentration of H^+ ions in the solution. The pH scale generally ranges from 0-14, where 7 is considered neutral.

pH is one of, if not the most, significant drivers of bacterial communities in soil (Lammel et al., 2018; Rousk et al., 2010). Broadly, microbes can be divided into three groups based on the pH when growth is optimal. Neutrophiles grow best at a pH between 5 and 9; most pasture grasslands are typically within this range. Acidophiles grow best at a pH of < 5 , whilst alkaliphiles prefer a pH above 9 (Jin & Kirk, 2018). As H^+ ions are directly involved in a number of cellular processes, such as energy production (ATP synthesis/hydrolysis), redox reactions, and extracellular enzyme function, microbes are extremely adapted to their ideal pH conditions with relatively low tolerances (Jin & Kirk, 2018; Rousk et al., 2010). Functioning even only 1.5 pH units outside of their adapted ranges can result in stress-induced reductions of microbial efficiency and halve their optimal growth rate (Jin & Kirk, 2018; Rousk et al., 2010).

Furthermore, pH can also alter nutrient availability to indirectly affect microbial growth and function (Jin & Kirk, 2018; Lammel et al., 2018). Positive ions (cations) needed for microbial function, such as calcium and magnesium, can be bonded to clay surfaces within the soil matrix. As pH declines and the quantity of H^+ ions increases, cations can be displaced and released from soil surfaces for microbial use (Lammel et al., 2018). While beneficial when essential nutrients are released at specific pH levels,

elements toxic to microbes, such as aluminium, can also be freed (Lammel et al., 2018). In addition to these contrasting effects, plant growth and carbon input, both of which contribute to microbial respiration, can also be inhibited or encouraged by changes to pH depending upon plant physiology (Lammel et al., 2018). Both these indirect and direct effects emphasise the complex and often contradictory nature of soil pH and its relationship with microbial respiration.

2.3 Controls on microbial respiration: Carbon availability

All soil organic carbon in soil is not similarly available to microbes for decomposition, it is often protected or ‘stored’ within the soil matrix (Dungait et al., 2012; Lützow et al., 2006). In general, there are three main mechanisms that can ‘stabilise’ carbon by restricting microbial access/ability for decomposition. These mechanisms are; occlusion through aggregation, mineral interactions, and biochemical complexity (Lützow et al., 2006; Six et al., 2002).

2.3.1. Occlusion through aggregation

Aggregation is the process by which particles of OM, clay particulates, plant roots and other soil biomasses of various sizes are grouped to form microaggregates and macroaggregates within the soil profile (Amézketa, 1999; Six & Paustian, 2014). These aggregates can provide physical protection of carbon, making it less vulnerable to microbial degradation. The binding processes that aid in the formation of aggregates include inorganic binding agents (clays, calcium (Ca^{2+}), iron (Fe^{3+}), and aluminium (Al^{3+}) cations, Fe and Al oxides), roots, soil fauna, soil microorganisms and environmental conditions (Amézketa, 1999).

Throughout time there have been many models of how aggregates form and store carbon within the soil. One of the first significant publications that proposed a hierarchical concept of aggregate formation was Tisdall and Oades (1982). In this study, Tisdall and Oades (1982) developed a theoretical framework of aggregate formation, which suggested different aggregating processes acted at different hierarchical stages of aggregation. They hypothesised that free primary particles and silt-sized aggregates were initially bound together by; humified OM, cation complexes and oxides, into microaggregates. In turn, these microaggregates were then bound into macroaggregates by roots and fungi.

Oades (1984) adapted this concept by suggesting that primary particles, including previously formed microaggregates, actually formed macroaggregates initially, and further microaggregation occurred within these macroaggregates after formation. Due to the short-term binding nature of roots and fungi, these microaggregates would be released once a breakdown of larger aggregates occurred, suggesting that microaggregation is the key to long-term carbon storage in soils. These released microaggregates (including old and newly formed) were then available to reform into new macroaggregates. Angers et al. (1997) provided evidence for this model by tracing the decomposition of $^{13}\text{C}^{15}\text{N}$ -labeled wheat straw through aggregation processes. They found that ^{13}C was initially accumulated in macroaggregates structures, which, over time, declined in further macroaggregate analysis. After this decline, the signature was then detected in the microaggregates indicating that indeed these microaggregates had formed within macroaggregates, capturing added ^{13}C , and were released once the larger aggregates broke down.

Subsequently, multiple studies have suggested additions or variations of this concept. A study by Six et al. (2000) developed a conceptual model to explain how disturbance of aggregates by tillage disrupts the stabilisation of carbon in microaggregates by breaking down macroaggregates before full formation has occurred.

Like with tillage, aggregation can be negatively impacted by land-use change, intensification, and other agricultural management practices such as crop rotation and irrigation (Wiesmeier et al., 2019). However, with increasing understanding of how these practices impact aggregation, and carbon protection, there is also great potential to improve aggregation processes within soil and increase carbon sequestration in managed systems (Abbas et al., 2020; Lal, 2004).

2.3.2. Adsorption on to mineral surfaces

Mineral surfaces in soils are provided by a wide variety of clays including, 1:1 and 2:1 clays, and hydrous-iron (Fe) and aluminium (Al) oxides in soil. These surfaces provide cation exchange sites where ligand exchange, polyvalent cation bridges, and weak interactions including van der Waal and H-bonding of carbon compounds can occur (Figure 2.3; Jackson et al., 2017; Lützow et al., 2006; Philippe & Schaumann, 2014; Saidy et al., 2012).

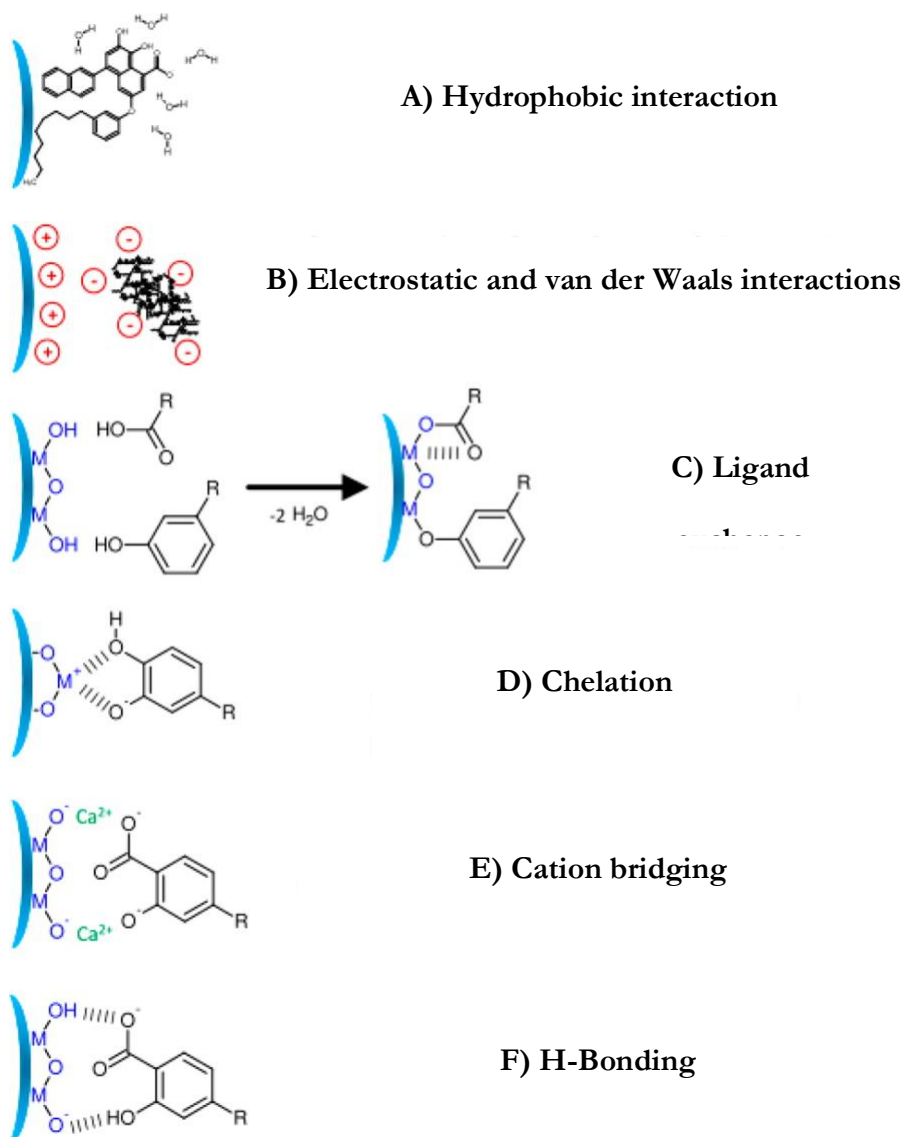


Figure 2.3: From Philippe and Schaumann (2014): “Schematic description of the diverse sorption mechanisms of DOM on the surfaces of colloids reported in the literature.”(p. 8948). Reprinted (adapted) with permission from Philippe and Schaumann (2014): Copyright (2020) American Chemical Society.

It has been widely noted that large proportions of carbon are strongly associated with fine silt and clay fractions in soil (Dungait et al., 2012; Kögel-Knabner et al., 2008; Lützow et al., 2006; McNally et al., 2018), and that increasing the amount of clay or Fe, Al-oxides soil can decrease microbial respiration in soil (Saidy et al., 2012). These observations give evidence to the hypothesis that these mineral interactions provide

strong protection of carbon substrates (Jackson et al., 2017; Kögel-Knabner et al., 2008; Six et al., 2002).

Different clays and metal oxides have varying specific surfaces areas, and therefore different capacities for sorption of substrates (Lützow et al., 2006; Philippe & Schaumann, 2014). The formation of these surfaces is highly influenced by climate, plant type and organic matter (OM) input, and interactions with these surfaces are sensitive to changes in pH, temperature (Lützow et al., 2006; Philippe & Schaumann, 2014).

2.3.3 Biochemical complexity

The complexity of carbon substrates is based on its molecular-level characteristics, including its elemental composition, the presence of certain functional groups (e.g. aromatic rings), and its molecular conformation, all of which influence their interactions with microbes and enzymes (Kleber et al., 2011; Lützow et al., 2006; Sollins et al., 1996). Diversity in substrate complexity can arise from the type of carbon input (i.e. plant litter, shoot exudates), from the creation of new products through microbial synthesis or depolymerisation in the soil (Sollins et al., 1996). Compounds that are considered more complex and require greater energy to decompose are considered recalcitrant, while labile compounds that are small in size and less complex are considered more accessible for microbial consumption (von Lützow et al., 2007).

While historically, biochemical complexity has been seen as the main factor for carbon stabilisation in soil, there is increasing research that questions this view (Lehmann &

Kleber, 2015). Substrates that are considered labile have been found in stable soil fractions with high carbon ages (Kleber et al., 2011; Schmidt et al., 2011). These findings have led to the hypothesis that if carbon compounds are available for consumption, they will be consumed, and other stabilisation factors are more important for carbon storage in soil (Dungait et al., 2012). Nevertheless, it still appears that substrate complexity is important in the early stages of decomposition before the effects of aggregation and mineral sorption take precedence (Lützow et al., 2006; Schmidt et al., 2011).

Because of the above three processes (Sections 2.3.1, 2.3.2 and 2.3.3), soil carbon exists as a range of compounds and materials, all of which exhibit different ages or mean residence times (MRT) within the soil. Due to the extensive nature of carbon in soils and their interactions, SOM is often fractionated into different pools based on how stable or resistant the carbon substrates in SOM are to decomposition (Cheng et al., 2007).

2.3.4 Conceptual models of SOM stabilisation

Multiple conceptual models have been developed over time to explain apparent soil carbon pools dynamics (Lehmann & Kleber, 2015). Although many models have been derived, their key concepts can be summarised by the four models below.

Humification model

The humification model is based on two carbon pools separated by molecular complexity. These two pools contain either small decomposable molecules or larger

complexed macromolecules, formed through the humification of the small molecules (Wershaw, 1993). Humic substances are thought to be carbon and nitrogen rich and increasingly resistant to decomposition. However, a lack of evidence on the formation pathways that lead to the creation of supposed humic compounds has caused disagreement about whether this synthesis actually occurs (Lehmann & Kleber, 2015).

Selective preservation

The selective preservation model assumes that organic inputs are comprised of labile and recalcitrant compounds (Sollins et al., 1996). Labile compounds are considered as compounds that are small in size and less complex making them easier for microbes to consume. Recalcitrant compounds are more complex and require greater energy inputs from microbes to decompose (von Lützow et al., 2007). Due to the increase in energy expenditure when consuming recalcitrant compounds, this model suggests microbes selectively degrade labile carbon over recalcitrant, leaving recalcitrant carbon in soil storage for longer.

Progressive decomposition

The progressive decomposition model can be described as an overall reduction in the energy required for decomposition over time (Trumbore, 1997). This reduction in required energy indicates that when degradable materials enter the soil, they are continuously decomposed by soil microbes into smaller and less complex compounds, which subsequently need less energy to decompose and continue on this energy-reducing path until the carbon is eventually released as CO₂ (Hedges et al., 2000). In terms of carbon stability, under this model long term SOM is likely to be made of more chemically recalcitrant compounds, which will take longer to break down to smaller,

easily consumed components. More recently, it has been shown that this is not necessarily true and less complex compounds often make up long term carbon storage in soil (Cotrufo et al., 2013; Dungait et al., 2012; Kleber et al., 2011).

Soil Continuum Model

Although both the progressive decomposition model and selective preservation model have merits, they do not fully encapsulate soil carbon dynamics. Both models focus on how consumable carbon fractions are, avoiding the complications brought on by other chemical, biological, and physical controls. Lehmann and Kleber (2015) suggested a soil continuum model (SCM) combining the top-down theory of the progressive decomposition model with interactions from aggregate formation and mineral surface adsorption. Overall, this model suggests that soil carbon stability occurs through greater adsorption of compounds onto colloid surfaces and increased incorporation into aggregates as compounds become molecularly smaller. However, adsorption and incorporation can occur at any compound size, and carbon stability is more fluid, dependent on outside influences (Section 3.2.2), and does not remain static in one stable pool. The SCM focused more on the positioning and availability of soil carbon within soil structure and its contribution to aggregate formation, rather than just the difficulty of compound consumption (Lehmann & Kleber, 2015).

MEMS Framework

Plant litter is commonly defined by its relative decomposability (**). Materials with low concentrations of chemically recalcitrant substrates decompose quickly, while highly recalcitrant materials are slower to deteriorate (Cotrufo et al., 2013). Despite apparently slower decomposition, in SOM, high recalcitrant compounds do not necessarily

accumulate and stabilise. Often products derived from labile constituents have been found in long term SOM rather than more complex materials (Cotrufo et al., 2013; Dungait et al., 2012; Kleber et al., 2011). The Microbial Efficiency-Matrix Stabilisation framework (MEMS) hypothesises that labile plant products are more utilised by microbes and are thus the dominant source of microbial products (Cotrufo et al., 2013). These products are then quickly stabilised in SOM through aggregation and the mineral soil matrix. This hypothesis aims to combine the inherent degradability of plant material with soil properties and may be an important theory to include in long term modelling. However, adding variability and components such as carbon use efficiency and C:N ratios does add complexity to modelling (Cotrufo et al., 2013).

2.4 Controls of microbial respiration: Temperature

All chemical reactions, including those involved in microbial respiration, are temperature dependant (Rogers, 2010). Microbial respiration is temperature dependant in two ways, firstly through indirect effects on the availability substrate, and secondly through direct effects on microbial metabolism and enzyme kinetics (Conant et al., 2011; Davidson & Janssens, 2006).

2.4.1 Substrate availability

Temperature dependence of substrate availability is dominated by OM-mineral interactions and physical occlusion of substrate (Conant et al., 2011; Davidson & Janssens, 2006). While temperature effects on occlusion are likely based on environmental effects on aggregation, which are harder to define, OM-interactions are governed by chemical reactions, and temperature response can be estimated based on

thermodynamic theory (Conant et al., 2011). OM-mineral interactions can be defined as either: i) fast, high-affinity (binding potential) reactions that are regulated by equilibrium thermodynamics; ii) slow, low-affinity reactions, governed by diffusion, or iii) strongly-bonded substrates (Conant et al., 2011; Nguyen et al., 2019).

High-affinity substrates

High-affinity substrates have a high binding potential and tend to form stronger, harder to break bonds. When under constant pressure and temperature, an equilibrium is established between the processes of adsorption and desorption of high-affinity substrates, which minimises the Gibbs free energy of the system (Conant et al., 2011; Rogers, 2010). Le Châtelier's Principle indicates that when a system is subjected to a change in temperature, concentration, volume or pressure, the equilibrium adjusts to minimise the stress. (Rogers, 2010). When temperature changes occur to a system in equilibrium, the equilibrium adjusts counteractively to minimise said temperature change. As adsorption is often an exothermic process while desorption is endothermic, an increase in temperature would cause the balance to shift towards the endothermic desorption of high-affinity substrate. In contrast, a decline in temperature would shift the equilibrium towards exothermic adsorption (Conant et al., 2011; Davidson & Janssens, 2006; Rogers, 2010; Wallenstein et al., 2011).

Low-affinity substrates

Low -affinity substrates have low binding potential, so in order for interactions to occur with OM-Surfaces, they must be in close proximity to said surfaces. Diffusion is most often the rate-limiting step in low-affinity substrate adsorption substrates (Conant et al., 2011; Nguyen et al., 2019). With increasing temperature, molecules gain

energy and movement, increasing the rate of diffusion (Conant et al., 2011; Rogers, 2010). Increased diffusion naturally increases the likelihood of substrate interaction with OM-surfaces and thus adsorption of low-affinity substrate (Conant et al., 2011; Nguyen et al., 2019).

In addition to this effect, as temperature increases, the desorption of high-affinity substrate is encouraged, leaving ‘holes’ on OM-surfaces for new bonds to occur. These ‘holes’ also increase the ability of low-affinity substrate to interact with OM surfaces (Conant et al., 2011; Davidson & Janssens, 2006).

Covalently bound SOM

Substrate interaction with OM-surfaces through covalent bonds are unlikely to respond, over a significant time frame, to temperature changes (Conant et al., 2011; Davidson & Janssens, 2006).

2.4.2 Microbial metabolism and extracellular enzymes

In order for microbes to assimilate carbon, larger weight molecular compounds must first be broken down into smaller constituents. This hydrolytic or oxidative process, facilitated by extracellular enzymes, is called depolymerisation (Conant et al., 2011; Wallenstein et al., 2011). Enzymes function by reducing the activation energy of reaction rates allowing complex reactions, which would ordinarily require high temperatures, to occur under *in situ* conditions (Hobbs et al., 2013; Wallenstein et al., 2011). Due to the necessity of enzymes for most biological processes, temperature controls on enzymes kinetics and microbial metabolism likely have a strong influence

on larger ecosystem responses, such as that of soil respiration (Alster et al., 2020; Schipper et al., 2014).

Enzyme kinetics

Enzymatic catalysis rates are a function of the concentration of enzymes, the enzyme kinetics and substrate availability, all of which are temperature dependant (Conant et al., 2011; Wallenstein et al., 2011). The specifics of enzyme kinetics involved in catalysed reactions are currently too difficult to measure but are suggested to be based on both the flexibility of an enzyme to bind and release molecules and the flexibility of the enzymes active site (Wallenstein et al., 2011). Based on thermodynamics, like all reactions, the rate of catalysis increases with temperature. However, at a certain point, rates decline to a temperature optimum (Hobbs et al., 2013; Schipper et al., 2014).

Historically, this attenuation of reaction rate has been attributed to the denaturation of enzymes at higher temperatures. Yet this phenomenon occurs at much lower temperatures than denaturation should occur (Alster et al., 2020; Wallenstein et al., 2011). More recently, a proposed theory models the response of enzyme rates based on changes in heat capacity (ΔC_p^\ddagger) which can be influenced by enzyme flexibility (Arcus et al., 2016; Hobbs et al., 2013; Schipper et al., 2014). This theory provides a more appropriate rationale for diminishing reaction rates with increasing temperature and will be discussed further in Section 2.5.

Microbial metabolism

The concentration of enzymes available is a balance between microbial production and enzyme turnover. When temperature increases, both these rates are likely to increase as the activation energy of these processes declines (Conant et al., 2011; Wallenstein et al., 2011). The rate of microbial production of enzymes and enzyme turnover is highly dependent on the type of microbes in the system, which can vary substantially spatially and temporally (Wallenstein et al., 2011). Additionally, as temperatures increase and activation energies lower, microbes may adjust and reduce the production of extracellular enzymes. Changes could also occur in the microbial community structure as a system adapts to temperature change over time (Bradford, 2013; Conant et al., 2011; Wallenstein et al., 2011). Shifts in community structure could influence what type of substrate is consumed, resulting in changes to catalytic rate or increases/decreases in community diversity all of which will alter how temperature affects the broader ecosystem (Bradford, 2013).

The controls of temperature on microbial respiration and the previous controls mentioned, are hard to individually distinguish in soil communities without focused experimental studies to look at community structure, determine catalytic rates, or identify physical soil properties (Wallenstein et al., 2011). While looking at individual enzymes or small scale communities, these types of experiments may be possible. However, scaling these to an ecosystem level with extensive spatial diversity is impractical. In order to understand how microbial respiration responds to controls such as temperature, robust models with theoretical underpinning are crucial to describing these larger complex systems.

2.5 Measuring the temperature response of microbial respiration in soil

As highlighted in the previous sections, microbial respiration is controlled by a multitude of variables, one of which is temperature. Temperature response itself is a result of many different processes that are temperature dependant within the soil.

As there is often confusion within the community over the terms temperature response, temperature sensitivity, and temperature dependence, the definitions that will be used in this thesis are outlined below.

- **Temperature dependence** is simply a statement of whether the rate of a process will be affected by changes in temperature. All chemical processes are a function of temperature, and therefore biological rates such as decomposition or respiration are also dependent on temperature (See section 2.4; Arcus et al., 2016; von Lützow & Kögel-Knabner, 2009; Wallenstein et al., 2011).
- **Temperature response** is the description of how a change in temperature affects the rate of a process. Models such as Arrhenius are commonly used to represent the response of chemical and biological rates with changing temperature (Fang & Moncrieff, 2001; Robinson et al., 2017). Temperature response and, by extension, temperature sensitivity can be **Intrinsic**, which relates to the inherent kinetic properties of a molecule's structure (Section 2.3.3). **Apparent** temperature response/sensitivity is the observed response under environment constraints (Sections 2.2 & 2.4; Davidson & Janssens, 2006).

- **Temperature sensitivity** is the rate of change with respect to temperature, or how fast/slow a change in rate occurs when all other variables are held constant (Alster et al., 2020; Sierra, 2012). Temperature sensitivity is typically calculated from temperature response models using either the first derivative or the ratio of rates over 10 °C, more commonly known as Q_{10} (Section 2.6; Alster et al., 2020; Sierra, 2012).
- **Substrate quality**, using thermodynamics, is based on the number of enzymatic steps, or the activation energy, required to release substrate as carbon dioxide (Bosatta & Ågren, 1999; von Lützow & Kögel-Knabner, 2009). High-quality substrates have lower activation energies and do not require as many steps for decomposition for microbes as low-quality substrates (von Lützow & Kögel-Knabner, 2009).

Multiple models have been used to describe temperature response and thus temperature sensitivity of microbial respiration; these include the Arrhenius and Lloyd and Taylor models, and more recently, macromolecular rate theory (MMRT).

2.5.1 Arrhenius

Arrhenius equation, developed in 1889, is based on the activation energy (E_a) of reactions, which is the energy peak that reactants must achieve in order to create products and describes the response of most abiotic chemical reactions to temperature (Equation 2.1; Hobbs et al., 2013; Schipper et al., 2014; Sierra, 2012). It is generally accepted that most reactions involved in decomposition, such as diffusion, sorption

and desorption, follow the Arrhenius equation and increase exponentially with response to temperature (Sierra, 2012).

$$k = Ae^{\frac{-E_a}{RT}} \quad \text{Equation 2.1}$$

Where k is the reaction rate constant, A is the frequency factor, E_a is the required activation energy of a reaction, R is the gas constant ($-8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T is the absolute temperature in Kelvin (Sierra, 2012; von Lützow & Kögel-Knabner, 2009).

In general, it can be stated that E_a is a measure of substrate quality. By definition, the Arrhenius equation shows that low-quality substrates (substrates that are more complex) with high activation energies have slower rates of decomposition, while those that are considered high-quality have faster rates (Sierra, 2012).

2.5.2 Lloyd and Taylor model

When observing residuals from multiple respiration data sets, Lloyd and Taylor (1994) found that the Arrhenius equation often overestimated respiration rates at high temperatures while underestimating rates at lower temperatures. To improve the data fits with varying temperatures, they suggested an empirical deviation from the Arrhenius equation (Equation 2.2; Fang & Moncrieff, 2001; Lloyd & Taylor, 1994).

$$R_s = Ae^{-E_0/(T-T_0)} \quad \text{Equation 2.2}$$

Where R_s is soil respiration rate, T is the temperature in K, $T_0 = 227.13$ K, A is a data-set dependent variable and $E_0 = 308.56$ K (Lloyd & Taylor, 1994).

While this model did provide a better fit than Arrhenius at higher temperatures, Fang and Moncrieff (2001) concluded it was still ineffective at lower temperatures. It is also important to note that there is no theoretical underpinning to the Lloyd and Taylor model.

Mixed models – DAMM

As mentioned, both the Arrhenius and Lloyd and Taylor models are exponential, meaning as temperature increases, rates of reaction also increase indefinitely (Sierra, 2012). Exponential increases are not true of biological processes, which show a decline in rate after a specific temperature is reached. In cases of enzyme-mediated reactions, when substrate is limited, rates of enzyme reactions do not occur at maximal rates (Davidson & Janssens, 2006; Wieder et al., 2015). Instead of following Arrhenius kinetics, Michaelis-Menten kinetics can be considered (Equation 2.3).

$$k = \frac{V_{\max} [S]}{(K_m + [S])} \quad \text{Equation 2.3}$$

Where k is the reaction rate, V_{\max} is the maximal rate of enzymatic activity, K_m is the Michaelis-Menten constant, representing the affinity of an enzyme for substrate (equal to substrate concentration at $V_{\max}/2$) and $[S]$ is the substrate availability (Davidson & Janssens, 2006).

For reactions, such as decomposition, which containing both enzyme processes and physical chemistry, model like the Dual Arrhenius and Michaelis-Menten kinetics model (DAMM; Davidson et al., 2012), which combine multiple factors, can be useful as they tend to rely more on theoretical processes rather than empirical representations of soil respiration (Davidson et al., 2012; Wieder et al., 2015). However, as the parameters in these models are based on individual microbial characteristics, when scaling to an ecosystem or global scale, it becomes increasingly difficult to adequately represent the enzyme kinetics (Wieder et al., 2015). Additionally, fluctuations in enzyme properties and population with temperature and time mean that even when combined, these models may not accurately represent microbial respiration or explain the observable decline in biological reaction rates (Davidson & Janssens, 2006).

2.5.3 MMRT

The macromolecular rate theory (MMRT) was first developed to model the observed temperature optimum of enzyme activity, not described by standard exponential models (Alster et al., 2020; Arcus et al., 2016; Hobbs et al., 2013; Schipper et al., 2014). Historically a decline in enzyme activity after a temperature optimum has been attributed to enzyme denaturation. However, these declines can occur well below temperatures required for denaturation. MMRT is a theoretical extension of the Arrhenius equation, which includes the thermodynamic properties of enzymes to predict a temperature optimum for enzyme-mediated reactions without denaturation (Alster et al., 2020; Arcus et al., 2016; Hobbs et al., 2013; Schipper et al., 2014).

The theory uses transition state theory, an expanded Arrhenius equation, in which activation energy (E_A) is equivalent to the change in Gibbs free energy (ΔG_p^\ddagger) between the unbound ground-state and the transition state, and Boltzmann and Planck's constants and a transmission coefficient (κ ; assumed here as 1) replace A (Equation 2.5; Arcus et al., 2016; Hobbs et al., 2013). ΔG_p^\ddagger is calculated from the difference between the change in enthalpy (ΔH_p^\ddagger) and change in entropy (ΔS_p^\ddagger) for the reaction (Equation 2.4; Arcus et al., 2016; Hobbs et al., 2013).

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad \text{Equation 2.4}$$

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta G^\ddagger}{RT} \quad \text{Equation 2.5}$$

Where k_B and h are Boltzmann and Planck's constants. ΔG_p^\ddagger is the change in Gibbs free energy between the ground state and transition state, R is the gas constant ($-8.314 \text{ J K}^{-1} \text{ mol}^{-1}$) T is the absolute temperature in Kelvin (Arcus et al., 2016; Hobbs et al., 2013).

One of the assumptions of the transition state theory is that ΔG_p^\ddagger is independent of temperature, which may be true for small molecules (Arcus et al., 2016; Hobbs et al., 2013; Schipper et al., 2014). However, biological processes are often mediated by enzymes which are large macromolecules with high heat capacities (C_p). Heat capacity can be defined as the temperature dependence of enthalpy (H) and entropy (S) and, by extension, the Gibbs free energy (G) of the system (Arcus et al., 2016; Hobbs et al.,

2013). The internal energy of a system is portioned between transitional, vibrational, rotational and electronic modes. When enzymes bind to a transition state, there is a reduction in the available vibrational and rotational modes resulting in a negative change in the heat capacity of the system (Arcus et al., 2016; Hobbs et al., 2013). By definition, a large negative change in heat capacity results in a change in the temperature dependence of the G (Equation 2.5).

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger = [\Delta H_{T_0}^\ddagger + \Delta C_P^\ddagger(T - T_0)] - T[\Delta S_{T_0}^\ddagger + \Delta C_P^\ddagger(\ln T - \ln T_0)]$$

Equation 2.5

Where T_0 is a reference temperature, $\Delta H_{T_0}^\ddagger$ and $\Delta S_{T_0}^\ddagger$ is the difference in enthalpy and entropy, respectively, ΔC_P^\ddagger is the difference in heat capacity between the ground state and transition state at constant pressure.

By incorporating equations 2.4 and 2.5, we get the base equation for the macromolecular rate theory (Equation 2.6).

$$\ln(k) = \ln\left(\frac{k_B T}{h}\right) - \frac{\Delta H_{T_0}^\ddagger + \Delta C_P^\ddagger(T - T_0)}{RT} + \frac{\Delta S_{T_0}^\ddagger + \Delta C_P^\ddagger(\ln T - \ln T_0)}{R}$$

Equation 2.6

If the MMRT is assumed, reactions mediated by enzymes with large heat capacities will result in the significant temperature dependence of G, and thus the temperature response of rates will deviate from Arrhenius behaviour. Conversely, if ΔC_P^\ddagger is zero, G will be independent of temperature, and temperature response of rates will appear as

a typical Arrhenius-like curve (Arcus et al., 2016; Hobbs et al., 2013; Schipper et al., 2014).

The use of ΔC_p^\ddagger and MMRT for biological systems allows for the determination of the temperature response of a reaction and the calculation of a temperature optimum (T_{opt}) and T_{inf} (inflection point; the temperature at which change in rate is maximal), with a reliable theoretical underpinning (Schipper et al., 2014). Additionally, MMRT has shown potential for applications at different spatial scales, from enzyme dynamics to multiple ecosystem processes and even global respiration modelling.

To date, MMRT has been applied to biological rates at a range of scales from enzymes and microbes up to complete soil and plant systems. Schipper et al. (2014) applied MMRT to a range of temperature response data from enzymes and soil processes such as denitrification, methane production and respiration and found that MMRT could predict temperature optimum for a wide range of microbial processes. Liang et al. (2018) modelled the temperature response leaf respiration and found consistent ΔC_p^\ddagger and T_{opt} across for leaf respiration from multiple biomes but a potential biogeographic pattern for measures of T_{inf} . In terms of soil microbial respiration, Robinson et al. (2017) applied the MMRT model to soil respiration derived from 5-hour incubations at 30+ individual temperatures along a temperature gradient. They found that soil type did not have a significant influence on ΔC_p^\ddagger , T_{opt} or T_{inf} on a farm scale. However, there may have been a slight variation in temperature response for soil collected in summer compared to collections during the rest of the year (Robinson et al., 2017).

More recently, Schipper et al. (2019), using the same methodology as Robinson et al. (2017), incubated soils from paired irrigated and non-irrigated sites to compare the temperature response of soil respiration under differently managed conditions. They found that irrigated soil had a significantly higher T_{opt} and T_{inf} and less total respiration than non-irrigated sites (Schipper et al., 2019). Lower respiration rates under irrigation were argued as indicative of less substrate available for microbial consumption. Lower availability, combined with higher T_{opt} and T_{inf} , lead to the hypothesis that substrate availability is a primary influence for the temperature response of soil respiration.

2.5.4 The Dual Control Hypothesis

The Dual Control Hypothesis suggests that observed temperature response results from a balance between soil processes that are governed by either Arrhenius kinetics or mediated by enzyme function with a clear temperature optimum (Figure 2.4; Schipper et al., 2019).

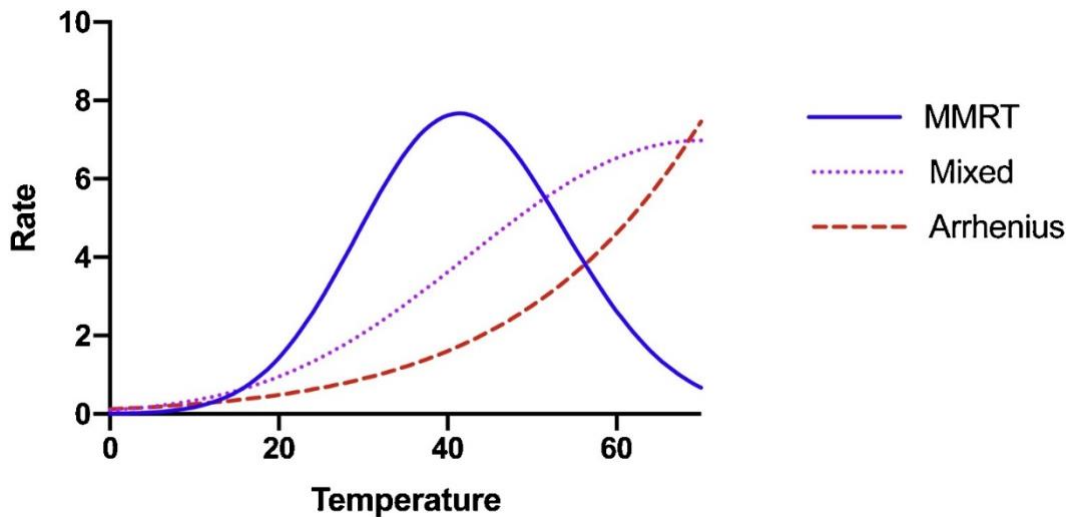


Figure 2.4: From Schipper et al. (2019): “A conceptual figure of the trade-offs between the response of enzymatic systems (following macromolecular rate theory) and the response of physical chemistry (following the Arrhenius function) with increasing temperature. The ‘mixed’ line depicts a combination of responses to temperature, where, as the biological system becomes more limited by substrate supply, the temperature response increasingly resembles the Arrhenius response” (p. 6).

In cases such as that of the irrigated soil from Schipper et al. (2019), where substrate is limited (reduced respiration rate). The rate-limiting steps for microbial respiration are processes, such as diffusion, sorption and adsorption, which control substrate release to microbes for consumption (Schipper et al., 2019). These processes are based on physical chemistry that has an Arrhenius response to temperature. Alternatively, when substrate is readily available for microbial decomposition, the rate-limiting steps are enzyme dynamics with MMRT-like responses to temperature and observable temperature optima. (Schipper et al., 2019).

The Dual Control hypothesis allows MMRT parameters, such as T_{opt} and T_{inf} , to be used not only be used as direct comparisons between the temperature response of various soils but to give insight into soil carbon dynamics and comparisons of substrate

availability under differing conditions, without the initial need for more complex analysis. However, more research is needed, using a wide range of soil and different carbon substrates, to determine the generality and applicability of this hypothesis.

2.5.5 Calculating temperature sensitivity of soil respiration

As mentioned previously, temperature sensitivity is the rate of change with respect to temperature, or how fast/slow a change in rate occurs when all other variables are held constant (Alster et al., 2020; Sierra, 2012). Temperature sensitivity is typically expressed in two ways: 1) absolute temperature sensitivity and 2) relative temperature sensitivity (Sierra, 2012).

Absolute temperature sensitivity

Absolute sensitivity represents the absolute change in some measure X (respiration flux, decomposition rate or turnover time) for a given unit change in temperature. More simply, it is the rate of a rate of change (Sierra, 2012).

Given the rate of a process modelled by the Arrhenius equation (Equation 2.2), to test for the absolute temperature sensitivity of soil respiration, the first derivative can be used (Equation 2.7). In this example, a homogenous substrate is assumed and thus $R_s = k C$.

$$\frac{\partial R_s}{\partial T} = \frac{\partial k}{\partial T} C = k \frac{E}{RT^2} C \quad \text{Equation 2.7}$$

Where R_s is respiration rate, T is the absolute temperature in Kelvin, R is the universal gas constant, k is the decomposition rate, E is the activation energy, and C is the amount of carbon available for decomposition (Sierra, 2012).

Relative temperature sensitivity

Relative temperature sensitivity expresses the change in some measure X by relative to the actual value of the measure X , or in basic terms, the ratio of rates (Sierra, 2012).

Again, given the rate of a process (R_s), modelled by the Arrhenius equation, Equation 2.8 can represent the temperature sensitivity of respiration in relative terms.

$$\frac{1}{R_s} \frac{\partial R_s}{\partial T} = \frac{E}{RT^2} \quad \text{Equation 2.8}$$

Relative temperature sensitivity can also be assessed using the Q_{10} function, which approximates the term E/RT^2 (Sierra, 2012). Q_{10} is the factor by which a reaction, such as soil respiration, changes in relation to a 10 °C increase in temperature (Davidson & Janssens, 2006; Sierra, 2012).

$$Q_{10} = \frac{R_{S_{T+10}}}{R_{S_T}} \quad \text{Equation 2.9}$$

Mathematically, the absolute and relative temperature sensitivity equations, $\partial R_s / \partial T$ and $(1/R_s) \partial R_s / \partial T$, have an inverse relationship and thus provide contradictory results with regards to temperature change and for substrate quality (Schipper et al., 2014;

Sierra, 2012). In absolute terms, high-quality substrates can be considered more temperature-sensitive, whilst in relative terms, low-quality substrates are more sensitive (Schipper et al., 2014; Sierra, 2012). Mathematically, the relative sensitivity of respiration is identical to the relative sensitivity of other reaction rates such as decomposition or turnover. In contrast, absolute sensitivity of respiration is a direct result of relative sensitivity for decomposition rate and total substrate availability (Equation 2.7) and thus is proportional to the size of the available carbon pool, which must be taken into account (Sierra, 2012).

There are multiple concerns to consider regarding the use of different measures of temperature sensitivity in the literature. Firstly, there is confusion in the literature over the absolute and relative terms and, therefore, the reporting of measure used (Sierra, 2012). As these two measures produce contradictory results, the distinction of what measure was used is critical to state when reporting data to ensure comparisons are drawn from similar measures. Additionally, understanding the context of the sensitivity measures is crucial (Sierra, 2012). Relative measures of temperature sensitivity are described in reference to the measure itself and cannot be confused as if it were relative to the pool size. Only absolute measures of respiration rate are related to carbon availability (Sierra, 2012). Finally, although the Arrhenius model was used in this example, multiple functions can be used to estimate Q_{10} . Different models can lead to drastically different estimates and introduce function biases (Davidson & Janssens, 2006; Sierra, 2012). Also, there are cases where functions have produced similar Q_{10} results to collected respiration data, using randomly generated data (Sierra, 2012).

While both measures of temperature sensitivity have pros and cons, consistency and reliable reporting of methods is required in the literature to allow better comparisons and a greater understanding of temperature sensitivity in general.

2.6 Identifying and measuring carbon pools

It is widely accepted that there are multiple pools of carbon within the soil matrix, based on the stability or resistance of carbon substrate to decomposition. At least two pools of carbon exist in the soil, which can be broadly defined as an active/ fast-cycling pool that is available for decomposition and a stored/slow-cycling pool. However, there are multiple methods of identifying and actively separating these carbon pools from the soil matrix to observe soil carbon dynamics. One approach for defining carbon pools is to use the mean residence time of the soil organic matter within the soil matrix. The turnover time can be calculated using the decay rate of a naturally occurring radioactive carbon isotope, ^{14}C (Larionova et al., 2015; Poeplau et al., 2018; von Lützow et al., 2007). Carbon substrates with longer residence times can be considered resistant to degradation or stored (von Lützow et al., 2007). In order to observe relationships between carbon storage and stabilisation mechanisms, the soil matrix can be separated into measurable pools based on a substrate's biogeochemical properties (von Lützow et al., 2007). There are multiple processes that can create separate carbon pools; however consistently obtaining identical pools, with homogeneous carbon mean residence times, for comparison is extremely difficult (von Lützow et al., 2007). It has also been suggested that stable carbon in SOM is not necessarily older than labile fractions, and thus using mean residence time as a measure of carbon stability is not realistic (Dungait et al., 2012).

Nevertheless, the primary methods used to separate carbon pools in soil can typically be grouped as either physical or chemical fractionation approaches. Physical fractionation typically is either through aggregate size fractionation, particle size fractionation or density fractionation (Poeplau et al., 2018). Chemical fractionations are separated using solubility extractions, hydrolysis, oxidation and fumigation techniques (Poeplau et al., 2018).

2.6.1 Aggregate fractions

Aggregate size is the simplest way to separate soil pools and is often applied as an initial separation step (Six et al., 2002). Dry and wet sieving of aggregates separates free SOM (active) from aggregate protected fractions. Aggregate fractionation is a very crude division of soil pools as many compounds in the free fraction may be protected in other ways.

2.6.2 Particle size

Particle size separation is based on the notion that different forms of SOM are associated with different particle sizes and compositions (Christensen, 1992). Sand fractions, due to a large proportion of un-reactive quartz, typically have low sorption of OM (active pool) compared to the clay fraction with its large surface area and strong negative charge (stable pool). Particle size is a very rough division of soil pools, with many theoretical flaws, yet this separation is not destructive to a soil's inherent chemical properties allowing for further testing of separated fractions (von Lützow et al., 2007).

2.6.3 Density

Particle density typically follows separation by particle size. Similarly, sized particles can have different densities, depending on the form of the protected carbon. Soil minerals are typically sorted into the light fraction, whilst organo-mineral complexes are much heavier (von Lützow et al., 2007). The most commonly used agent for density fractionation is sodium polytungstate, as it can be used to form many different densities and is nontoxic, unlike previously used agents (Christensen, 1992). Again, like aggregate and particle size, density is not a thorough and accurate separation of soil carbon pools due to the complexity of soil carbon interactions.

2.6.4 Salt extractions

The simplest chemical fractionation methods are extractions using a salt solution (KCl, K₂SO₄, NaOH) or water to remove likely labile compounds that are soluble/dissolve in the selected solutions (Poeplau et al., 2018; Prentice & Webb, 2010). An active carbon pool would dissolve into the salt/alkaline solution whilst the more stable humic substances would precipitate out. This technique was initially used to identify humic substances for the humification model (Section 2.3.4). However, it is widely debated that this reaction, in reality, causes the precipitation of the new ‘stable’ substances rather than merely separating them from an active pool. In addition to this, the alkaline method rarely extracts more than 50% of organic carbon, leading to inconsistent results (Lehmann & Kleber, 2015).

2.6.5 Hydrolysis extractions

Hydrolysis extractions are used to isolate individual compounds which may be biologically available, based on their functionality or composition, i.e. Humic Acid (Helfrich et al., 2007; Poeplau et al., 2018). Multiple steps involving various acid/base solutions are often applied to separate SOC into two pools: the hydrolysate (active) and the residue (stable) (Helfrich et al., 2007; Poeplau et al., 2018).

Several studies have shown that the hydrolysate fractions are much younger than the bulk SOC whilst the residue was much older (von Lützow et al., 2007), indicating this is a moderately accurate representation of available and stable pools. Nevertheless, this method only identifies the size of two carbon pools in soil samples and does not allow for further investigation into the decomposition and movement of carbon between these pools (von Lützow et al., 2007).

2.6.6 Oxidation extractions

Oxidation extractions follow similar procedures to hydrolysis extractions but use oxidative solutions (NaOCl , H_2O_2) to isolate potentially recalcitrant compounds for further testing (Helfrich et al., 2007; Poeplau et al., 2018). Oxidation extractions are designed to more closely imitate natural enzyme processes to break down soil compounds (Helfrich et al., 2007; Poeplau et al., 2018). Like with hydrolysis, oxidation extractions often come at the risk of potentially altering the chemical structure of carbon compounds. Also, there is no guarantee that the methods are produce comparative results within the literature as many different extraction techniques using

multiple solutions and different digestion lengths exist (Poeplau et al., 2018; Prentice & Webb, 2010).

2.6.7 Soil fumigation

Microbial biomass is arguably the most important carbon pool in soil or at least the most studied. Microbial biomass not only regulates other potentially stable carbon pools but is also considered a large active part of the labile carbon (von Lützow et al., 2007). Soil fumigation, most often with chloroform, can be used to estimate microbial biomass carbon (von Lützow et al., 2007).

2.6.8 ^{13}C analysis

The main problem with chemical and physical fractionation methods is that there is no guarantee resulting carbon pools have not been artificially altered. Isotopic tracers (^{13}C and ^{14}C) can be used to separate functional pools of carbon without disrupting internal soil processes (Zacháry, 2019). Isotopic tracers can be artificially added to SOM using pulse-chase, or continuous, ^{13}C or ^{14}C labelling of photosynthesising plant material (Paterson et al., 2009). These types of experiments can investigate carbon allocation by plant growth in the soil and the consumption rates and storage of labelled carbon by soil microbes (Zacháry, 2019). Natural changes in isotopic values in the environment can also be traced with precise methodology (Paterson et al., 2009; Zacháry, 2019). C3 - C4 plant transition over time can cause a measurable shift in isotopic value (average shift of -19 ‰). This change in isotopic value can be used to separate older carbon with an isotopic value similar to the dominant plant species

before a transition, from the newer carbon inputs within soil to observe long-term carbon dynamics in soil (Larionova et al., 2015; Zacháry, 2019).

Isotope incorporation into the soil through plant roots creates a distinct isotope signature in soil organic material and subsequent CO₂ production. This signature can potentially be used to partition respired CO₂ into its sources of either ‘new’ carbon or SOC. Each of the methods mentioned above come with advantages and disadvantages for measuring the source of soil respiration (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019). Pulse-labelling with either ¹³C or ¹⁴C provides information on the direct pathways of plant allocation of carbon within soil and subsequent soil respiration at one point in time (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019). However, as it is impossible to entirely remove root and hyphae in the field or before incubation based experiments, measured respiration from labelled carbon is a combination of microbial decomposition and residual autotrophic respiration (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019).

Continuous labelling has a more homogenous distribution of label throughout a soil profile and allows for analysis of long-term carbon budgets rather than immediate allocation (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019). However, like pulse-labelling, it is difficult to remove autotrophic respiration from CO₂ and thus cannot observe changes to the microbial decomposition of different carbon pools. Additionally, it is a significantly more expensive and difficult methodology meaning it is only applied in limited situations (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019). Finally, while natural abundance techniques are simple, inexpensive, and does not require isotopic labelling, however, C3-C4 shifts

in plant type only provide a small difference in isotopic ratio requiring precise measurements of isotope ratio to observe statistical changes (Cheng & Gershenson, 2007; Paterson et al., 2009; Zacháry, 2019).

Overall the choice of isotopic labelling method is crucial for the analysis of microbial decomposition of soil carbon; however, the method of isotopic measurement of respired CO₂ is equally important.

Isotope Ratio Mass Spectroscopy

Isotope ratio mass spectrometry (IRMS) is the most commonly used method for analysing quantities of stable isotopes. By ionising a sample and accelerating the ions over a high potential, IRMS instruments create a stream of ions separated by their mass to charge ratio, i.e. lighter ions bend more than heavier ions. These now separated ions are measured using Faraday cups or a multiplier detector (Muccio & Jackson, 2009).

One disadvantage to IRMS is that gas samples must be purified before entering the mass spectrometer to allow only one species to enter at a time. Samples can be purified or separated using a single or a combination of traps, filters, catalysts and gas chromatography adding extra processing time and cost to the analysis (Muccio & Jackson, 2009). The cost of IRMS analysis has led to the use of laser absorption spectroscopy (LAS) as a cheaper but equally reliable analysis choice.

Tunable diode laser absorption spectroscopy

Tunable diode laser absorption spectroscopy (TDLAS) is the most commonly used form of LAS and is used extensively to identify concentrations of gaseous species (Bolshov et al., 2015; Paldus & Kachanov, 2005). It can detect these concentrations in the order of part per billion and additionally can determine temperature, pressure, velocity and mass flux if required. The primary method of TDLAS uses a Tunable diode light source, which is bounced off optics to an absorbing material with a detector. When a gaseous material that absorbs light at a specific wavelength is placed within the cavity, the diode is tuned over the known absorption wavelengths of the species causing a measurable reduction in light intensity hitting the detector (Bolshov et al., 2015; Paldus & Kachanov, 2005). This reduction can then be used to calculate the properties of the gas in question.

Although commonly used, the main disadvantage with LAS is it relies on the detection of a very small signal change, within a large background, including noise created by the equipment, (e.g. diode, detector) which severely limits its sensitivity (Bolshov et al., 2015; Paldus & Kachanov, 2005). To increase the sensitivity of the TDLAS method, either the absorption must be enhanced, or the noise reduced.

In order to increase absorption, the Cavity Ringdown Spectroscopy (CRDS) technique uses an optical cavity allowing the laser to be reflected back and forth between mirrors, making multiple passes through the gas until decayed (Baer et al., 2002; Paldus & Kachanov, 2005). When a gaseous material that absorbs light at a specific wavelength is placed within the cavity, the light is diminished faster compared to the empty cavity and can be recorded and used to calculate the concentration of the absorbing material

(Paldus & Kachanov, 2005). Although this technique does dramatically increase the sensitivity of the TDLAS technique, it requires the wavelengths to be in resonance with each other as they pass back and forward. The instrument, therefore, requires precise wavelength control and monitoring, making the machines more complex, less robust and more expensive to make and maintain (Baer et al., 2002; Paldus & Kachanov, 2005).

The Off-axis Integrated Cavity Output Spectroscopy (OA-ICOS) method was created to address the highlighted issues and reduce the cost of analysis. By placing the laser off centre, the beams of light do not interact and thus can be non-resonant. Non-resonance removes the need for precise wavelength control and overall increases the robustness of the instrument and reduces its vulnerability to vibration or temperature changes (Barker et al., 2011; Beinlich et al., 2017; Paldus & Kachanov, 2005). This method has been shown to produce reproducible and accurate results for both carbon and oxygen isotopes leading to a wide range of potential uses. In addition to this, as it is relatively low-cost and fast, its use can also be extended for low-income projects or research conducted at sites with minimal technological capabilities (Barker et al., 2011; Beinlich et al., 2017).

2.7 Temperature response of different carbon pools

Accepting that there are multiple pools of carbon, regardless of fractionation method, leads to the question, do all carbon pools respond in the same manner to changes in temperature?

There is a reasonable amount of literature reporting on the temperature response or sensitivity of at least two broad carbon pools in soil. From a synthesis of 30 + papers, von Lützow and Kögel-Knabner (2009) identified four contrasting conclusions often made within the literature:

- 1) Within the range of 5 – 35 °C, decomposition rates of stable SOM pools were not temperature sensitive.
- 2) Pools containing stable SOM have a higher temperature sensitivity of decomposition than pools containing labile SOM.
- 3) Labile and stable SOM pools respond similarly to changes in temperature.
- 4) The decay rate of stable SOM is not temperature sensitive, while the decay rate of labile SOM is very temperature sensitive.

Within the sampled papers, various techniques of fractionation, warming/incubation methods, and data analysis were used to measure the temperature sensitivity of soil respiration. However, even with the inconsistencies in measuring, modelling and reporting temperature response of soil respiration highlighted in this chapter, and high variability in reported Q_{10} , a majority of studies viewed by von Lützow and Kögel-Knabner (2009) supported the second conclusion that stable SOM pools are more temperature-sensitive than labile carbon. A similar synthesis of 50+ papers by Conant et al. (2011) agreed with the observations of von Lützow and Kögel-Knabner (2009), concluding that in general, SOM had a higher Q_{10} than the comparable labile pool. It is important to note that a majority of papers in this study were the same as those viewed in Lützow and Kögel-Knabner (2009), and thus, a similar result was somewhat to be expected. More recently, Wang et al. (2019) examined differences in observed temperature sensitivity in an extensive comparison of 81 studies encompassing

multiple ecosystems and different soil factors such as total nitrogen, clay content or soil organic carbon (SOC). Wang et al. (2019) found considerable variation in Q_{10} with different experimental methods and among ecosystems. In general, once again viewed studies concurred that the stable/recalcitrant carbon pool was more temperature-sensitive than the labile fraction (Wang et al., 2019).

2.7.1 Methodology concerns

Although it appears the literature tends to agree on the temperature sensitivity of stable and labile carbon. There is no absolute consistency in the measuring and reporting of temperature response of soil respiration or decomposition. In addition to this, many of the incubation based studies examined by von Lützow and Kögel-Knabner (2009), Conant et al. (2011), and Wang et al. (2019) use a limited number of incubation temperatures (~2-6 temperatures), and for extended incubation periods (7-700 days). Methods that employ long-incubation periods can experience changes to soil properties such as total carbon and nitrogen with time, altering observed temperature sensitivity. Likewise, microbial biomass and community structure have the potential to adapt to warmed conditions over long incubation periods (Bradford, 2013). Similarly, methods that fit complex functions to small data sets and derive response/sensitivity can be subjected to significant error. Robinson et al. (2017) showed that confidence in model fits deteriorates when using fewer than 20 sample temperatures demonstrating that as the number of data points declines, it becomes easier to fit any desired model and get seemingly reasonable results.

2.8 Temperature response of carbon pools under managed soils

This thesis examined the temperature response of respiration from new ^{13}C -labelled carbon sources and the effects of seasonal irrigation on the accumulation or subsequent cycling of these new carbon inputs. ^{13}C enriched soils were obtained from Carmona (2020) for analysis.

2.8.1 Changes in carbon allocation/storage under irrigation

For pastoral grasslands, irrigation is a crucial management practice used to increase food production (Trost et al., 2013). Irrigation is well known to alter both carbon inputs and outputs from the soil and thus is likely to alter carbon storage and potentially soil pool dynamics (Trost et al., 2013). It is widely accepted that applying irrigation increases above-ground biomass in pastoral systems, and for some cases, irrigation has also been shown to result in a decline of root biomass while also increasing microbial decomposition of soil carbon (Schipper et al., 2013; Scott et al., 2012; Trost et al., 2013). How this increased plant biomass, enhanced microbial respiration, and overall increased water content affected carbon stabilisation within the soil remains uncertain. In New Zealand, there is a heavy reliance on summer irrigation to maintain productivity, and recently, a substantial increase in usage throughout the country. Despite this dependence on irrigation, there is limited New Zealand specific data on carbon storage under irrigation (Mudge et al., 2017; Scott et al., 2012).

Of the New Zealand based studies, losses total carbon have under irrigation by multiple studies (Condon et al., 2014; Kelliher et al., 2012; Mudge et al., 2017;

Schipper et al., 2013), gains were found by Laubach and Hunt (2018), and no change (Kelliher et al., 2015) have all been reported by papers. Of these papers, three studies that showed losses came from the same long-term (70+ year) irrigation treatments at Winchmore Irrigation research station (Canterbury, New Zealand).

The Winchmore Trial was established in 1947 to investigate the management of the seasonal irrigation and fertiliser application of sheep-grazed pasture. Simply put, two separate experiments were established; a fertiliser experiment consisting of the application of either 0, 250, or 376 kg ha yr⁻¹; and an irrigation experiment consisting of non-irrigated and border-dyke irrigation where 100 mm of irrigation was applied when soil reached either 10 or 20% gravimetric water content. Kelliher et al. (2012) measured total carbon storage under the long-term dryland and 20% irrigation treatments. They reported a significant decrease in total carbon stock under irrigation management compared to non-irrigated sites and estimated a 36% increase in carbon inputs (litter fall) and a 97% increase in annual losses through respiration (Kelliher et al., 2012). Schipper et al. (2013) used historical cores to measure decadal changes in total carbon and nitrogen under varying irrigation (dryland and 20% treatments) and nutrient regimes at also at Winchmore. They found above ground production was greater under irrigation, but total root mass was reduced, resulting in a lower total soil carbon content (0-10 cm) within the first ten years of irrigation application (Schipper et al., 2013). Condon et al. (2014) observed increased pasture production (74%), respiration rates, and significantly less SOC within the first 1m of soil at sites that received frequent irrigation (20% treatment compared to dryland). The C:N ratio in the SOC light fraction (< 1.8 g cm⁻³) at these sites suggested wetter conditions accelerated decomposition of this more labile carbon fraction (Condon et al., 2014). However, there were no significant differences between the 10 % irrigation and

dryland treatments (Condrón et al., 2014). Overall, these three studies came to similar conclusions regarding the potential mechanisms of carbon loss. It was suggested increased leaching of carbon and increased decomposition under irrigation likely drive losses of carbon from sheep-grazed pasture systems. However, as these studies are all from the same experiment, they cannot represent the spatial diversity of New Zealand pasture systems.

A comprehensive study by Mudge et al. (2017) measured changes in carbon stocks under irrigation by sampling 34 paired irrigated and non-irrigated sites across New Zealand (ranging from 3 – 90 years of seasonal irrigation application). They observed in the top 0.3 m of soil, an average of 6.99 t C ha⁻¹ less carbon under irrigation compared to adjacent dryland sites. They came to a similar conclusion to the above studies that increased water content encourages leaching of existing soil carbon and may increase microbial decomposition leading to a decline in soil carbon over time (Mudge et al., 2017).

In contrast to the above losses of carbon, Kelliher et al. (2015) observed no change in the carbon content in soils under irrigated treatments, depending on sampling depth. When comparing the SOC concentration from a seasonally irrigated dairy farm (established for 11 years) to a nearby, un-irrigated site (Lincoln, New Zealand), they found that within the first 0.3 m of soil, carbon content was 28% higher under irrigation. However, when comparing the first 0.8 m of soil, there was no statistical difference between sites (Kelliher et al., 2015). They highlighted how sampling depth and different calculations could affect the reported carbon content, leading to inconsistent comparisons within the literature (Kelliher et al., 2015).

Additionally, one short-term study found gains of carbon in soils under irrigation. Laubach and Hunt (2018) used a carbon balance approach to assess changes to carbon stocks in soils under intensively managed dairy and dryland pastures. Over three years, they found that SOC in irrigated pasture soils increased by $\sim 0.81 \text{ Mg ha}^{-1}$; however, yearly changes declined when stocking rates increased. Longer-term effects of irrigation on carbon stocks were not determined in this study.

While the studies mentioned above may provide insights into total soil carbon stock changes, the mechanisms of reported losses or gains of SOC are often only hypothesised. How carbon is allocated and transferred between different carbon pools within a soil profile, and how this changes under different management practices is difficult to determine (Jackson et al., 2017).

Carmona (2020) used ^{13}C -CO₂ enrichment of new photosynthate carbon inputs to soil in two different experiments to observe the allocation, and subsequent cycling, of carbon under seasonal irrigation. The first experiment involved labelling photosynthate carbon with ^{13}C during the three-month application irrigated or dryland treatments, followed by a chase period where the moisture contents were kept the same (Carmona, 2020). This experiment allowed the observation of changes to new carbon storage during the irrigation period and subsequent cycling of this new carbon. Overall, irrigation did not affect the amount of new carbon stored in soil when compared to dryland soils, but when separated into soil fractions, more new carbon was found in the fine particulate organic matter (POM; 53–250 μm) and clay ($< 5 \mu\text{m}$) fractions under the irrigation treatment Carmona (2020).

The second experiment by Carmona (2020) photosynthate carbon, labelled under the same spring moisture content, followed by three months seasonal irrigation and dryland conditions, then a further six months where the moisture contents were kept the same. They observed no significant differences in total new carbon recovered after three months of irrigated and dryland treatments; however, the carbon allocation throughout the soil profile differed. After the application of a dryland treatment, soils had more new carbon present in the non-rhizosphere soil than previously irrigated soils, a significant proportion of which was recovered from the stable clay fraction. Additionally, more new carbon recovered the lower profile (15–25 cm) in soils treated with seasonal irrigation. Overall the increased movement of carbon into the lower profiles and stable fractions was hypothesised to be caused by increased biological activity under irrigated treatments increasing root turnover, aggregation, and potential short-term stabilisation of carbon under irrigated pasture. (Carmona et al., 2020).

2.8.2 Temperature response of soil respiration under irrigation

New Zealand based studies on the temperature response of soil respiration under irrigation is also lacking in the literature. Schipper et al. (2019), as mentioned previously, the measured temperature sensitivity of paired irrigated and non-irrigated soils finding that soils under irrigation have less total respiration and were more temperature sensitive (Q_{10}) than the non-irrigated soils. This study made suggestions on the stability of soil carbon based on total respiration (Section 2.5), yet further investigation is required to fully understand carbon transfers between different labile and stable carbon pools within a soil profile and whether these are stabilised or destabilised due to management changes (Jackson et al., 2017).

2.9 Summary

This chapter has highlighted how further investigation is needed into the temperature response of microbial respiration, with particular regard to different respiration derived from different carbon pools. Microbial respiration is a complex biological process involving multiple variables, each of which are individually temperature dependent. While there may be a consensus that the temperature sensitivity of the broadly defined stored carbon pool is higher (Q_{10}) than the active pool in soil. Inconsistencies in the knowledge of basic definitions, understanding of biochemical processes, the use of multiple fractionation methods, and temperature response models demonstrate the need for a new protocol for measuring temperature response/sensitivity of soil respiration.

Of the fractionation methods researched, isotope analysis, particularly ^{13}C , is still an underutilised tool for measuring the temperature response of multiple carbon pools in soil. This chapter has highlighted recent advances in the effectiveness and cost reduction of isotope analysers situation. There is potential for using both artificial tracers and natural shifts in $^{13}\text{C} / ^{12}\text{C}$ to trace carbon from plant roots, into soil storage and out through microbial respiration.

Finally, there is a lack of knowledge on carbon storage and the temperature response carbon pools under irrigated pastoral systems. Increasing knowledge of how carbon is cycled under these systems and how this responds to temperature increases would be important given the reliance and prevalence of irrigation in New Zealand.

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Chapter 3: Contrasting temperature responses of soil respiration derived from soil organic matter and added plant litter.

Jasmine M. Robinson^{a*}, Shaun L. L. Barker^b, Vickery L. Arcus^a, Samuel R. McNally^c, Louis A. Schipper^{a*}

^aThe University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand

^bARC Centre of Excellence in Ore Deposits (CODES), School of Natural Sciences (Earth Sciences), University of Tasmania, Private Bag 79, Hobart, TAS 7001

^cPlant & Food Research, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140

The contributions of authors were (see co-authorship form at back of thesis):

Jasmine Robinson, Louis Schipper and Shaun Barker decided on experimental design. Sam McNally contributed enriched litter for analysis. Jasmine Robinson ran experiments, analysed data, completed statistical analysis and was responsible for writing the manuscript. Louis was the primary reviewer for this manuscript with Shaun Barker, Vick Arcus and Sam McNally providing additional comments.

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3.1 Abstract

Accurate description of temperature response and sensitivity of different carbon pools within soil is critical for accurately modelling soil carbon stocks and changes. Inconsistent sampling, incubation and fractionation methods highlights the need for new approaches to this area of study. We developed and tested a new protocol which allowed measurement of the temperature response of two carbon pools within soil. A Horotiu silt loam soil, wet up to 60% maximum water holding capacity, was mixed with ^{13}C enriched plant litter and incubated for 5 or 20 hours, at 30 discrete temperatures ($\sim 2 - 50\text{ }^{\circ}\text{C}$). A mixing model was used to separate respired CO_2 into litter and soil organic matter sourced carbon pools, which were then fitted using macromolecular rate theory. Overall, litter sourced respiration had a low T_{opt} and was less temperature sensitive (Q_{10}) than soil organic matter sourced respiration, which was more Arrhenius-like. We attribute these differences in temperature parameters to the factors that control the availability of carbon to microbes from the labile litter (enzyme kinetics with a clear temperature optimum) compared to the relatively stable soil organic matter (desorption and diffusion that exhibit Arrhenius behaviour). The developed method is rapid and reliable and may be suited to exploring temperature response of a variety of ^{13}C -labelled pools in soil and more clearly demonstrates that labile carbon has very different temperature response than more stable carbon pools in soil.

3.2 Introduction

Changes in global soil carbon stocks will be directly and indirectly influenced by how organic matter decomposition responds to changes in global temperature (Conant et al., 2011). Currently, many carbon cycle models partition soil organic matter (SOM) into one or more pools and apply first-order linear decay rates to describe transfers between pools and the atmosphere (Bradford et al., 2016; Luo et al., 2016; Wieder et al., 2015). These simple equations are then adjusted for environmental properties such as temperature or rainfall. However, these models likely do not fully represent the role of the microbial response to changes in temperature, moisture, and substrate accessibility (Luo et al., 2016; Wieder et al., 2015). It is generally assumed that decomposing communities will behave similarly in response to warming changes, with the same ability to access carbon. Still, inconsistencies in definitions of carbon pools and methodological approaches can hamper efforts to determine a unifying theory (Conant et al., 2011; Luo et al., 2016; Wieder et al., 2015). Increasing our understanding of decomposition temperature sensitivity for different pools of soil carbon is essential for improving the modelling of carbon cycling and therefore reducing uncertainty in long-term projections of stocks (Lefèvre et al., 2014; Todd-Brown et al., 2012).

While there is broad agreement that different pools of carbon within soil may have distinct responses to temperature, definitions of these pools vary widely. The challenges associated with how to define carbon pools, what these pools contain, and at times confusing terminology, can lead to inconsistent conclusions on their temperature sensitivity (Abramoff et al., 2017; Lehmann & Kleber, 2015). When theoretically defining multiple carbon pools, the mean residence time of SOM within the soil matrix can be calculated using the decay rate of a naturally occurring radioactive

carbon isotope, ^{14}C (Larionova et al., 2015; Poeplau et al., 2018; von Lützow et al., 2007). Carbon compounds with longer ^{14}C residence times are reasoned to be more resistant to degradation (von Lützow et al., 2007). Functionally however, it remains difficult to consistently separate the soil matrix into distinct pools with homogeneous mean carbon residence times, to reveal potential relationships between carbon storage, stabilisation mechanisms, and sensitivity to environmental changes (von Lützow et al., 2007).

There are multiple methodologies used to measure the size of functional carbon pools. These approaches can typically be grouped under the terms of physical and chemical fractionation. Physical fractionation occurs using aggregate size fractionation, particle size fractionation or density fractionation (Poeplau et al., 2018). Physical fractionation aims to separate carbon pools based on physical protection, either through aggregate formation or clay interactions (Dungait et al., 2012; Jackson et al., 2017; Poeplau et al., 2018). Chemical fractionations are generally achieved via solubility extractions, hydrolysis, oxidation and fumigation techniques and separate carbon pools by digesting soil to retain specific classes of molecules (i.e. Humic acid) or to mimic enzyme degradation (Helfrich et al., 2007; Poeplau et al., 2018). In addition to chemical and physical fractionation, isotopic tracers (^{13}C and ^{14}C) can be used to separate functional pools of carbon, without direct separation of soil constituents. Isotopic studies typically observe the turnover time of SOM within soil and can distinguish various carbon sources of respired CO_2 (Zacháry, 2019). In some cases, experiments use sites where ^{13}C inputs have changed associated with changes in vegetation from C3 to C4 plants. Alternatively, ^{13}C or ^{14}C labelled substrates (pure carbon or through labelling of plant biomass) can be added to soil (Trumbore & Zheng, 1996; Zacháry, 2019).

While there is a wide range of fractionation techniques reported in the literature, we have focused on two widely accepted, broad-defined pools of carbon in soil. Firstly, an active pool that is readily available/soluble and can be decomposed easily. Secondly, a stored pool that is unavailable due either to physical protection, chemical bonding or structural complexity, all of which need additional influences for the carbon to be solubilised and consumed (Dungait et al., 2012; Lehmann & Kleber, 2015; Schmidt et al., 2011). Accepting at least two carbon pools in soil, we arrive at the as yet unresolved question of “Do these pools behave the same way in response to changing environmental variables or disturbances?”. One key response that is critical for predicting carbon cycling in soil is the temperature sensitivity of microbial decomposition from different carbon pools. (Conant et al., 2011; von Lützow & Kögel-Knabner, 2009). Many studies have reported the temperature sensitivity of decomposition of two apparently distinct carbon pools using various fractionation techniques and field/laboratory methodologies (most often reported as changes in Q_{10}).

von Lützow and Kögel-Knabner (2009) summarised the current knowledge of temperature sensitivity of differing carbon pools based on 30+ papers with various techniques of fractionation, warming/incubation methods, and data analysis. They identified four contrasting conclusions often made within the literature:

- 1) Within the range of 5 – 35 °C, decomposition of stable SOM pools was not temperature sensitive.
- 2) Stable SOM pools have a higher temperature sensitivity of decomposition than that of labile SOM pools.
- 3) Both labile and stable SOM pools respond similarly to changes in temperature.

- 4) The decay rate of labile SOM was very temperature sensitive, but not that of the stable SOM.

A majority of studies at the time supported the second conclusion although with high variability in Q_{10} observed, particularly among ecosystems. Similarly, a synthesis by Conant et al. (2011) also found most papers (50+ papers sampled, but also drawn from a similar pool of studies) concluded that the stable pool of SOM had a higher temperature sensitivity (higher Q_{10}) than the labile pool. They concluded that the overall temperature response of soil was likely controlled by three aspects of SOM decomposition, all of which are individually temperature dependant. These aspects are the depolymerisation of carbon (the degradation of molecules into smaller decomposable fractions), the rate of enzyme production, and substrate availability. Studies that can separate the response of these factors will be instrumental in the understanding of soil temperature dependence (Conant et al., 2011). More recently a large comparison of 81 papers by Wang et al. (2019) examined differences in observed temperature sensitivity over multiple ecosystems and whether different soil factors such as total nitrogen, clay content or soil organic carbon (SOC) were important. They found there was considerable variation in Q_{10} with different experimental methods and among ecosystems, but in general, studies suggested the stable/recalcitrant carbon pool was more temperature sensitive than the labile fraction (Wang et al., 2019).

In addition to the inconsistencies in the pool partitioning methods utilised, many of the incubation based studies examined by von Lützow and Kögel-Knabner (2009), Conant et al. (2011), and Wang et al. (2019) use limited number of incubation temperatures (~2 - 6 temperatures), and for extended incubation periods (7 - 700 days).

Long-incubation periods may alter temperature sensitivity as soil properties such as total carbon, nitrogen and microbial biomass change with time. Furthermore, Robinson et al. (2017) showed that confidence in model fits deteriorates when using fewer than 20 sample temperatures. Fitting complex functions to small data sets and deriving sensitivity is susceptible to substantial error. When the number of data points declines, it becomes easier to fit any desired model, allowing many different, model-dependent conclusions regarding temperature sensitivity. A lack of consistency in model choice is not ideal as if, as the current majority suggests, a large stable pool of carbon is very temperature sensitive, we may misrepresent the degree to which decomposition will be further accelerated under climate warming. If we cannot predict these effects with some consistency, it becomes more difficult to usefully inform future climate-change related policy (Bradford et al., 2016; Zhou et al., 2018).

A critical choice in making predictions about future temperature response of soil respiration is selection of appropriateness of fitted model. Macromolecular rate theory (MMRT) is a relatively new model that captures the observed initial exponential increase in rate but then curves to fit an optimum, a phenomenon seen often in biological responses to temperature but not always fully accounted for in more commonly used models i.e. Arrhenius, Lloyd and Taylor (Alster et al., 2020; Schipper et al., 2014). The MMRT model is based on the hypothesis of the changing of the heat capacity during enzyme catalysis with increasing temperature, providing a theoretical background for model fitting and potentially additional measures for temperature sensitivity which may be more intuitive for comparison between treatments (Alster et al., 2020).

Our objective was to develop and test a protocol that would allow rapid and accurate determination of the temperature response of two pools of carbon in soil. It was critical that this system allowed for measurements to be made within 5 h to reduce opportunity for microbial adaptation had not occurred, and at more than 20 different temperatures so temperature response could be adequately described. We combined our previous approach for incubation of soils at ~40 temperatures (Robinson et al., 2017) with a newly developed method for measurement of the ^{13}C content of acidified carbonates (Barker et al., 2011), adapted to measure respired carbon dioxide (CO_2). We illustrate the utility of this approach by measuring soil respiration of soil with added ^{13}C labelled litter, simultaneously at 30 temperatures, ranging between $\sim 2 - 50^\circ\text{C}$. Total respired CO_2 was then partitioned into litter-derived respiration, and soil carbon derived CO_2 using an isotope mixing mode and temperature response determined by fitting MMRT to the individual curve. Successful development of such an approach may allow future tracing of ^{13}C using photosynthetic enrichment, or natural $^{13}\text{C}/^{14}\text{C}$ shifts to measure temperature sensitivity of different soil C pools.

3.3 Methods

Total respiration measurement

Measurement of the temperature response of soil respiration is commonly conducted by incubating samples at multiple temperatures, allowing CO_2 to accumulate in the headspace, which is then measured by infra-red gas analysis or gas chromatography analysis. Here, we adapted a method previously described by Robinson et al. (2017), which allows for rapid (5 h) measurement of soil respiration (from 4 g of soil in 28 ml Hungate tubes) at 30+ temperatures along a temperature gradient within a large

aluminium block, heated at one end and cooled at the other (ranging from $\sim 2 - 50^{\circ}\text{C}$ to $\sim 1.5^{\circ}\text{C}$ increments). A large number of independent temperatures reduces uncertainty in model fitting, improving comparisons between soils and management practices (Robinson et al., 2017; Schipper et al., 2019). In this study, we extended this approach to measure $\delta^{13}\text{C}$ of respired CO_2 using a modified, off-axis integrated cavity output spectrometer (OA-ICOS) instrument (Los Gatos Research, model 908-0021), as described by Barker et al. (2011); Beinlich et al. (2017).

The first experiment focussed on determining whether incubation time (5 and 20 hours) altered temperature response of respiration. Being able to conduct an experiment with a 5 h incubation time would minimise potential adaptation, an important process but not the focus of this study. For method development and testing, we mixed a well-drained, allophanic, Horotiu soil (Typic Udivitrang; 8.5% C, 0.83% N, soil pH of 6.0, $\delta^{13}\text{C}_{\text{VPDB}}$: -26.5 ‰) wet up to 60% maximum water holding capacity (MWHC), with ^{13}C enriched, air-dried litter (Ryegrass/clover, $\delta^{13}\text{C}_{\text{VPDB}}$: 140 ‰), ground using a mortar and pestle. Above-ground plant litter was labelled using isotopic, pulse labelling techniques described by McNally et al. (2016). MWHC was determined using the method described by Harding and Ross (1964). Litter (~ 0.005 g) was weighed out and added to soil (~ 4 g) in 30 Hungate tubes (28 ml), 3 times for replication. Tubes were stoppered, crimped, shaken to distribute litter, and then immediately incubated for 5 h or 20 h on a temperature gradient block. A longer incubation time (20 hours) was tested for comparison (also 3 replications) to 5 h incubations, as some soils respired inadequate CO_2 for ^{13}C analysis after only 5 h.

After incubation, the tubes were immediately placed in ice to minimise additional CO₂ production and then stored overnight at -20 °C. Within 24 h, headspace gas samples were analysed using an OA-ICOS analyser for $\delta^{13}\text{C}$ as well as total CO₂. For samples with anticipated high concentration of total CO₂, headspace gas samples (1 ml) were removed prior to OA-ICOS for analysis on an infra-red gas analyser (IRGA) (see below), given the limited range of CO₂ standards used for the OA-ICOS (Robinson et al., 2017).

In addition to using two incubation durations, a second experiment used three soil moisture contents (30, 60 and 80%, MWHC, 3 replications each) to test whether soil moisture altered soil respiration, or its temperature response, from different carbon sources.

¹³C analysis method

To measure ¹³C using the OA-ICOS, outlet and inlet needles were inserted into the headspace of Hungate tubes containing the incubated soils. The carrier gas (scrubbed nitrogen gas, N₂) was pumped through the inlet needle into the Hungate tube to mix with respired CO₂ then drawn into the outlet needle and the measurement cavity, using the applied vacuum from the OA-ICOS instrument. Custom modifications allowed internal values to be controlled using an external computer running LabVIEW® software (Barker et al., 2011). The sample gas (at ~20 °C) passed through an analysis cell, and the CO₂ concentration and isotopic composition were continuously recorded. For the OA-ICOS to precisely measure ¹³C content, the concentration of gas inside the analysis cell must stay constant (at ~200 ppm) for ~1 min. To accomplish this, the valves which control gas flow through the cell were triggered to close once the

concentration of CO₂ reached a pre-determined value (~250 ppm) after peak CO₂ was reached. Once closed, trapped gas (now at ~200 ppm) was then analysed for the concentrations of ¹²C ¹⁶O ¹⁶O, ¹³C ¹⁶O ¹⁶O and ¹²C ¹⁸O ¹⁶O by the OA-ICOS. Analysis occurred for one minute to obtain a precise isotope reading (internal standard error of ± 0.07 ‰) for each gas sample, at approximately the same concentration to remove the influence of variable CO₂ concentration on the measured isotopic ratios (Barker et al., 2011). Trapping of CO₂ at 200 ppm was chosen as the desired concentration for analysis after initial trials demonstrated that concentrations lower than 160 ppm were increasingly inaccurate due to insufficient sample within the analysis cell for spectral fitting to reliably occur. A higher concentration (up to 2500 ppm) could also be used, but the low temperature soil incubations in this study often produced insufficient CO₂ for higher concentration analysis.

To ensure accurate ¹³C measurements, ¹³C values from sample CO₂ were adjusted to $\delta^{13}\text{C}$ relative to Vienna Pee Dee Belemnite (VPDB) using the averaged values of replicated standards. Three replicates of five known $\delta^{13}\text{C}_{\text{VPDB}}$ standards were analysed, spanning the predicted range of ¹³C values from samples. These standards were both international reference materials (NSB-1: 1.95 ‰, sigma: -14.18 ‰, BDH: -24.95 ‰, as reported in Beinlich et al. (2017), and IAEA-303a: 94 ‰ and 303b: 460 ‰), and internal carbonate standards (range of bicarbonate solutions from ~ 30 ‰ to 250 ‰, standardised using the international reference carbonate standards). Along with standards, a drift correction was measured every ten samples by analysing a 1500 ppm CO₂ reference gas for one minute. Standard deviation of standards showed an average error of ± 0.5 ‰, per experimental analysis. As every treatment in this study had three

repeats of each CO₂ sample, it was assumed that any measurement error would be incorporated into the standard error calculated between sample repeats at a later stage.

Data Analysis

Total CO₂ produced was divided into litter derived (enriched), and soil derived components using a 2-pool mixing model (Equation 3.1).

$$f = \frac{(C_S - C_R)}{(C_L - C_R)} \quad \text{Equation 3.1}$$

Where C_S, C_R, and C_L are the $\delta^{13}\text{C}_{\text{VPDB}}$ value of the soil, the respired CO₂ and the added litter respectively. Partitioning resulted in three temperature-respiration curves (a) combined soil + litter, (b) litter-derived, and (c) SOM-derived respiration rate.

The temperature responses of the resulting separate respiration rates (Rs) were fitted using MMRT (Equation 3.2) (Hobbs et al., 2013; Schipper et al., 2019). The temperature optimum (T_{opt}) and inflection point (T_{inf}) were calculated using Equation 3.3 and Equation 3.4. T_{opt} is the temperature at which respiration is maximal and the change in respiration rate is equal to zero. T_{inf} is the point at which the change in respiration rate is greatest (Schipper et al., 2014). Two metrics of temperature sensitivity were also calculated, including the first derivative (rate of change of the respiration curve) and Q₁₀ (ratio of rates of respiration measured 10 °C apart).

$$\ln(R_s) = \ln \left[\frac{k_B}{b} \right] - \frac{\Delta H_{T_0}^\ddagger + \Delta C_p^\ddagger (T - T_0)}{RT} + \frac{\Delta S_{T_0}^\ddagger + \Delta C_p^\ddagger (\ln T - \ln T_0)}{R} \quad \text{Equation 3.2}$$

$$T_{opt} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger - R} \quad \text{Equation 3.3}$$

$$T_{inf} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger \pm \sqrt{-\Delta C_p^\ddagger R}} \quad \text{Equation 3.4}$$

Where ΔC_p^\ddagger (J mol⁻¹ K⁻¹) is the change in heat capacity between the enzyme-substrate complex and the enzyme bound to the transition state, k_B is Boltzmann's constant, and b is Planck's constant. In the case of soil respiration, the ΔC_p^\ddagger is the approximate ensemble mean ΔC_p^\ddagger of all the enzymes involved in respiration (Schipper et al., 2014). $\Delta H_{T_0}^\ddagger$ is the change in enthalpy (J mol⁻¹), and $\Delta S_{T_0}^\ddagger$ (J mol⁻¹ K⁻¹) is the change in entropy between the enzyme-substrate complex and the enzyme bound to the transition state at a reference temperature (T_0 , 298 K, 25 °C).

Statistical Analysis

All statistical analyses and data processing were performed using MATLAB 2017a (The MathWorks Inc., Natick, MA, USA). We used a two-way ANOVA to test for differences in parameters between incubation times and between moisture contents in general. Tukey's honest significant difference test was used post hoc to compare parameters between incubation times and among moisture contents. One-way ANOVA was used to compare carbon source parameters within a treatment

(incubation time or moisture content), the same post hoc analysis compared parameters between two specific carbon sources within a treatment.

3.4 Results

As expected, total respiration rate generally increased with increasing temperature with a decline in the increase of the rate of CO_2 production at higher temperatures for total respiration (Tot-Rs) (Figure 3.1, a). This reduction was most pronounced above 35 °C, as seen in litter-sourced respiration rate (Lit-Rs) (Figure 3.1, b). However, SOM-sourced respiration rate (SOM-Rs) often produced no apparent temperature optimum when fitted with MMRT. In these cases, the resulting fit is functionally no different than Arrhenius (ΔC_p^\ddagger is indistinguishable from 0) and does not allow the determination of a T_{opt} , T_{inf} or ΔC_p^\ddagger .

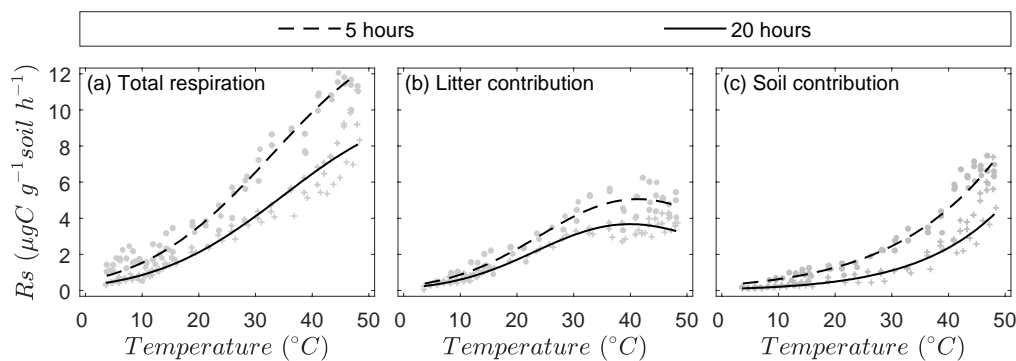


Figure 3.1: Respiration rates (Rs) from a Horotiu soil (60% MWHC) mixed with ^{13}C enriched litter, incubated for either 5 h or 20 h. Total respiration (a) was partitioned into respiration from litter (b) and bulk soil organic matter (c) using a mixing model. All were fitted using the MMRT model. Symbols indicate 3 replications of data for 5 h (filled circle) and 20 h incubations (plus). Different lines represent average response for different incubation lengths.

In the first experiment comparing incubation length, values of T_{inf} for Tot-Rs varied around 34 – 37 °C, whilst Lit-Rs T_{inf} was between 23 – 24 °C for both the 5 h and 20 h incubations (Table 3.1). Again, SOM-Rs generally exhibited an Arrhenius response that did not allow for determination of these parameters. Data points from all three replications for each incubation time were included in Figure 3.1 as a demonstration of the spread of data, and how MMRT was fit to the data.

Table 3.1: Calculated MMRT parameters (mean \pm std, n= 3) derived from respiration of a Horotiu soil mixed with an ^{13}C enriched litter.

Incubation time*	C source	T _{opt} (°C)	T _{inf} (°C)	ΔC_p^\ddagger (J mol ⁻¹ K ⁻¹)	$\Delta H_{T_0}^\ddagger$ (J mol ⁻¹)	$\Delta S_{T_0}^\ddagger$ (J mol ⁻¹ K ⁻¹)	Rs at 10 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)	Rs at 20 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)	Rs at 30 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)
5 h ^{A, A, A}	Total	62 \pm 11 ^a	34 \pm 4 ^a	-1344 \pm 576 ^a	43227 \pm 6425 ^a	-87 \pm 21 ^a	1.5 \pm 0.4 ^a	3.5 \pm 0.5 ^a	6.5 \pm 0.5 ^a
	Litter	42 \pm 1 ^b	24 \pm 1 ^b	-2611 \pm 759 ^a	40064 \pm 8692 ^a	-101 \pm 29 ^a	0.9 \pm 0.3 ^{ab}	2.3 \pm 0.3 ^b	4.1 \pm 0.4 ^b
	Soil	n/a	n/a	n/a	n/a	n/a	0.6 \pm 0.1 ^b	1.3 \pm 0.1 ^c	2.5 \pm 0.2 ^c
20 h ^{A, A, A}	Total	63 \pm 8 ^a	36 \pm 3 ^a	-1337 \pm 359 ^a	46285 \pm 4587 ^a	-80 \pm 16 ^a	0.9 \pm 0.1 ^a	2.1 \pm 0.2 ^a	4.1 \pm 0.6 ^a
	Litter	41 \pm 3 ^b	23 \pm 1 ^b	-2806 \pm 694 ^b	40085 \pm 4469 ^a	-103 \pm 16 ^a	0.6 \pm 0.1 ^b	1.7 \pm 0.2 ^a	3.0 \pm 0.4 ^a
	Soil	n/a	n/a	n/a	n/a	n/a	0.2 \pm 0.04 ^c	0.5 \pm 0.1 ^b	1.1 \pm 0.4 ^b
C source		< 0.001	< 0.001	0.005	0.233	0.168	0.016	0.002	< 0.001
Time		0.990	0.702	0.799	0.682	0.879	0.013	< 0.001	< 0.001
Source \times Time		0.806	0.617	0.783	0.686	0.734	0.209	0.061	0.033

Respiration (Rs, $\mu\text{gC g}^{-1} \text{h}^{-1}$) was sampled at the end of both a 5 and 20 h incubation period. Different letters (i.e., a, b, c) note the significant differences (< 0.05) between carbon source, within an incubation time. Significance level (p-value) given of main effects and interaction shown at base of table.

*Different letters (i.e. A, A, A) note the significant differences (< 0.05) for pairwise comparison of T_{opt}, T_{inf} and ΔC_p^\ddagger , respectively, between incubation times.

Examination of the 5 h incubation data revealed that the difference in T_{opt} and T_{inf} between Tot-Rs than and Lit-Rs was significant. T_{opt} and T_{inf} for Tot-Rs were also significantly higher than Lit-Rs in 20 h incubation data, with ΔC_p^{\ddagger} also being significantly different. When comparing the same parameters derived from different incubation times, we found no significant differences, apart from the respiration rates with 5 hour respiration rates being greater than 20 h rates. Additionally, no significant interaction between carbon source (total or litter) and incubation time was observed for the same parameters, further indicating any significant differences were due to carbon source rather than incubation time.

From the MMRT fits produced, temperature sensitivity (calculated as the first derivative and as Q_{10}) was derived, as shown in Figure 3.2.

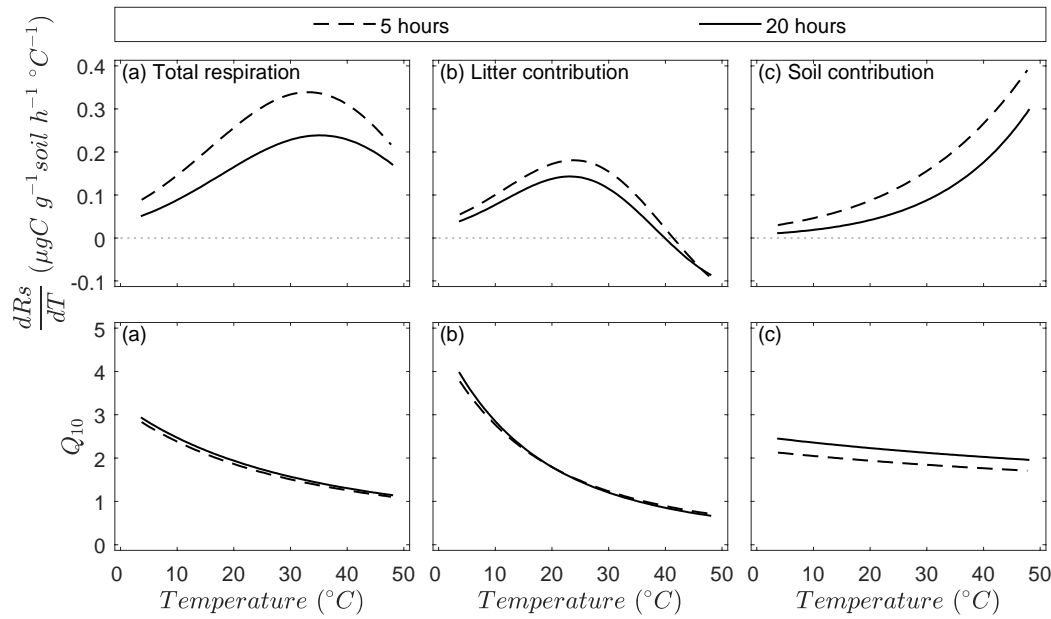


Figure 3.2: First derivative (dR_s/dT) and Q_{10} calculated from MMRT model fits of respiration rates from a Horotiu soil (60% MWHC) mixed with ^{13}C enriched litter, incubated over 5 h and 20 h. Total respiration has been partitioned into respiration from litter and bulk soil organic matter. Different lines represent average temperature sensitivity ($n = 3$) for different incubation lengths.

These measures were compared at 10, 20 and 30 °C in Table 3.2. For both incubation periods, dR_s/dT was significantly lower for SOM-Rs than Lit-Rs at lower temperatures. The highest dR_s/dT values for Lit-Rs was at 20 °C after which change in rate declined, SOM-Rs continued to increase with increasing temperature. Q_{10} for Lit-Rs was higher than SOM-Rs at 10 °C after both incubation times. At 20 °C and 30 °C, Q_{10} for Lit-Rs was lower, significantly so at 30 °C. Both these measures suggest litter decomposition was more temperature sensitive at lower temperatures, and this declined with increasing temperature (Table 3.2).

Table 3.2: Calculated Q_{10} and dRs/dT (mean \pm std, n= 3) derived from MMRT fits of respiration of a Horotiu soil mixed with an ^{13}C enriched litter.

Incubation time	C source	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dRs/dT at 10 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 20 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 30 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)
A, A, A5 hours ^{a, a, a}	Total	2.38 ± 0.44^a	1.86 ± 0.17^a	1.51 ± 0.03^a	0.14 ± 0.02^a	0.26 ± 0.02^a	0.33 ± 0.02^a
	Litter	2.76 ± 0.69^a	1.79 ± 0.22^a	1.24 ± 0.02^b	0.10 ± 0.02^b	0.18 ± 0.02^b	0.16 ± 0.03^b
	Soil	2.05 ± 0.15^a	1.94 ± 0.10^a	2.12 ± 0.09^c	0.05 ± 0.01^c	0.09 ± 0.01^c	0.16 ± 0.01^b
A, A, B20 hours ^{b, b, b}	Total	2.47 ± 0.29^a	1.94 ± 0.12^a	1.57 ± 0.03^a	0.09 ± 0.01^a	0.16 ± 0.03^a	0.23 ± 0.04^a
	Litter	2.81 ± 0.42^a	1.78 ± 0.11^a	1.21 ± 0.07^b	0.08 ± 0.01^a	0.14 ± 0.03^a	0.11 ± 0.02^b
	Soil	2.36 ± 0.11^a	2.23 ± 0.10^b	2.12 ± 0.09^c	0.02 ± 0.01^b	0.04 ± 0.01^b	0.09 ± 0.03^b
C source		0.076	0.012	< 0.001	< 0.001	< 0.001	< 0.001
Time		0.446	0.098	0.002	< 0.001	< 0.001	< 0.001
Source \times Time		0.836	0.218	0.002	0.218	0.150	0.200

The significance level of the main effects (p-value) and interaction shown at base of table. Significant differences (< 0.05) between carbon source and within an incubation time, are noted by different letters (i.e., a, b, c). * Significant differences (< 0.05) from pairwise comparison of Q_{10} at 10, 20 and 30 °C (uppercase, left side), and dRs/dT at 10, 20 and 30 °C (lowercase, right side) respectively, between incubation times are noted by different letters (i.e. A, A, A).

Q_{10} values were not significantly different between different carbon sources or incubation times at 10 °C, but there was a detectable difference at 20 and 30 °C. dRs/dT was significantly different for both carbon source and incubation time. The observed differences between incubation times were likely due to an increase in total respiration rate between the 5 and 20 h incubations which altered the magnitude of the rate and as a consequence dRs/dT .

In addition to incubation length, three soil moisture contents were also analysed in a second experiment to determine whether results differed in extreme moisture conditions (Figure 3.3). Tot-Rs increased with increasing temperature, as expected. At 30% MWHC, respiration rate was considerably lower than other moisture contents and incubation experiments. Lower CO_2 production led to a reduction of available data points for fitting MMRT, as more samples did not reach the minimum concentration required for $\delta^{13}C_{VPDB}$ analysis (Table 3.3).

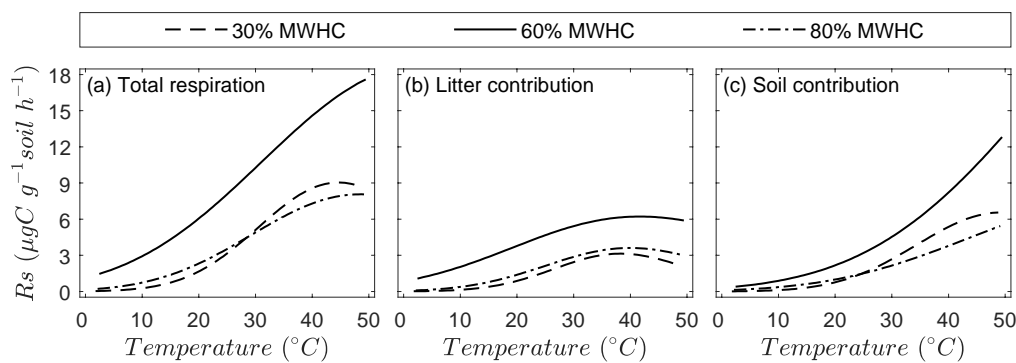


Figure 3.3: Respiration rates (R_s) from a Horotiu soil at three separate moisture contents (30%, 60% or 80% MWHC), mixed with ^{13}C enriched litter and incubated for 5 h. Using a mixing model, total respiration (a) was partitioned into respiration from litter (b) and bulk soil organic matter (c). All were fitted using the MMRT model. Different lines represent average response ($n = 3$) for different moisture contents.

Table 3.3: Calculated MMRT parameters (mean \pm std, n= 3) derived from respiration of a Horotiu soil at 3 different moisture contents, and an ^{13}C enriched litter.

Moisture Content*	C Source	T _{opt} (°C)	T _{inf} (°C)	ΔC_p^\ddagger (J mol ⁻¹ K ⁻¹)	$\Delta H_{T_0}^\ddagger$ (J mol ⁻¹)	$\Delta S_{T_0}^\ddagger$ (J mol ⁻¹ K ⁻¹)	Rs at 10 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)	Rs at 20 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)	Rs at 30 °C ($\mu\text{gC g}^{-1}\text{soil h}^{-1}$)
30%A, A, A	Total	45 \pm 2 ^a	31 \pm 1 ^a	-4228 \pm 611 ^a	80284 \pm 6466 ^{ab}	34 \pm 22 ^{ab}	0.3 \pm 0.1 ^a	1.7 \pm 0.1 ^a	5.1 \pm 0.2 ^a
	Litter	39 \pm 1 ^b	26 \pm 1 ^b	-5411 \pm 1034 ^a	70410 \pm 9791 ^a	-5 \pm 33 ^a	0.2 \pm 0.1 ^b	0.9 \pm 0.1 ^b	2.4 \pm 0.3 ^b
	Soil	49 \pm 1 ^c	34 \pm 1 ^c	-3797 \pm 214 ^a	90066 \pm 3460 ^b	61 \pm 11 ^b	0.1 \pm 0.1 ^b	0.8 \pm 0.1 ^b	2.7 \pm 0.1 ^b
60%B, A, B	Total	67 \pm 16 ^a	35 \pm 7 ^a	-1053 \pm 411 ^a	36690 \pm 2447 ^a	-103 \pm 8 ^a	2.9 \pm 0.3 ^a	6.0 \pm 0.2 ^a	10.2 \pm 0.2 ^a
	Litter	43 \pm 6 ^a	19 \pm 1 ^b	-1601 \pm 584 ^a	24326 \pm 2488 ^b	-150 \pm 8 ^b	2.0 \pm 0.3 ^b	3.8 \pm 0.2 ^b	5.4 \pm 0.2 ^b
	Soil	99 \pm 7 ^b	59 \pm 4 ^c	-751 \pm 91 ^a	51825 \pm 1492 ^c	-61 \pm 5 ^c	0.9 \pm 0.1 ^c	2.2 \pm 0.1 ^c	4.5 \pm 0.1 ^c
80%*C, A, C	Total	49 \pm 5 ^a	30 \pm 3 ^a	-2382 \pm 207 ^a	53152 \pm 5965 ^a	-56 \pm 18 ^a	0.8 \pm 0.2 ^a	2.4 \pm 0.5 ^a	5.0 \pm 0.7 ^{ab}
	Litter	40 \pm 2 ^a	25 \pm 2 ^a	-3643 \pm 60 ^b	52675 \pm 9232 ^a	-62 \pm 29 ^a	0.4 \pm 0.2 ^a	1.4 \pm 0.4 ^a	2.8 \pm 0.6 ^a
	Soil	72 \pm 15 ^a	44 \pm 10 ^a	-1322 \pm 368 ^c	56251 \pm 2923 ^a	-53 \pm 9 ^a	0.3 \pm 0.1 ^a	1.0 \pm 0.1 ^a	2.1 \pm 0.2 ^b
C source		< 0.001	< 0.001	0.001	0.008	0.009	< 0.001	< 0.001	< 0.001
Moisture		0.001	0.103	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Source \times Moisture		0.009	0.006	0.618	0.292	0.266	< 0.001	< 0.001	< 0.001

Respiration (Rs, $\mu\text{gC g}^{-1} \text{h}^{-1}$) was sampled at the end of a 5 hour incubation period. Moisture contents were 30, 60 and 80% MWHC. Different letters (i.e., a, b, c) note the significant differences (< 0.05) between carbon source, within a moisture content. For the main effects and interactions, significance level (p-value) is shown at base of table.*Different letters (i.e. A, A, A) note the significant differences (< 0.05) for pairwise comparison of T_{opt}, T_{inf} and ΔC_p^\ddagger , respectively, between moisture contents.**Values include only 2 reps, one was excluded as an outlier due to unrealistic parameters.

For all of the parameters examined in Table 3.3, carbon source and moisture content were significant factors determining differences in respiration values, and there was, in some cases, evidence of interaction between these two variables. Pairwise comparisons showed that T_{opt} and ΔC_p^\ddagger were significantly different among the three moisture contents. At 60% MWHC Lit-Rs and SOM-Rs were significantly different at all three temperatures, this was not the case at 30 and 80% MWHC. T_{opt} and T_{inf} were significantly different between Lit-Rs and SOM-Rs at 30 and 60% MWHC, while ΔC_p^\ddagger was significant at 80%.

Q_{10} declined with increasing temperature across moisture contents and carbon source. dR_s/dT increased with increasing temperature (Figure 3.4), apart from in the cases of Lit-Rs at 60 and 80% MWHC where a decline was seen at 30 °C (Table 3.4).

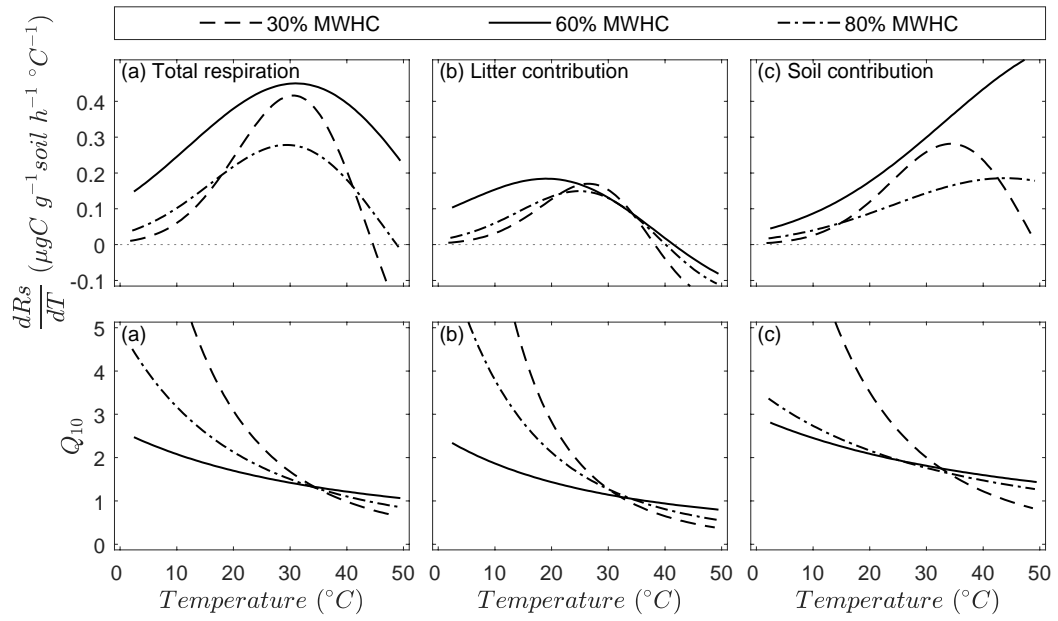


Figure 3.4: First derivative (dR_s/dT) and Q_{10} calculated from MMRT model fits of respiration rates from a Horotiu soil at three separate moisture contents (30%, 60% and 80% MWHC) mixed with ^{13}C enriched litter, incubated over 5 h. Total respiration has been partitioned into respiration from litter and bulk soil organic matter. Different lines represent average response ($n = 3$) for different moisture contents

Table 3.4: Calculated Q_{10} and dRs/dT (mean \pm std, $n=3$) derived from MMRT fits of respiration of a Horotiu soil, at three moisture contents, mixed with an ^{13}C enriched litter.

Moisture Content*	C source	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dRs/dT at 10 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 20 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 30 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)
A, A, A30% ^{a, a, a}	Total	6.20 \pm 1.14 ^a	3.08 \pm 0.27 ^a	1.68 \pm 0.06 ^a	0.06 \pm 0.01 ^a	0.24 \pm 0.02 ^a	0.41 \pm 0.04 ^a
	Litter	6.50 \pm 1.95 ^a	2.71 \pm 0.36 ^a	1.28 \pm 0.07 ^b	0.03 \pm 0.01 ^b	0.13 \pm 0.01 ^b	0.15 \pm 0.03 ^b
	Soil	6.65 \pm 0.51 ^a	3.51 \pm 0.16 ^b	2.00 \pm 0.06 ^c	0.02 \pm 0.01 ^b	0.12 \pm 0.01 ^b	0.26 \pm 0.01 ^c
B, B, B60% ^{b, b, a}	Total	2.06 \pm 0.2 ^{ab}	1.70 \pm 0.06 ^a	1.44 \pm 0.05 ^a	0.24 \pm 0.01 ^a	0.37 \pm 0.03 ^a	0.45 \pm 0.02 ^a
	Litter	1.89 \pm 0.2 ^a	1.44 \pm 0.05 ^b	1.15 \pm 0.06 ^b	0.15 \pm 0.02 ^b	0.18 \pm 0.03 ^b	0.13 \pm 0.01 ^b
	Soil	2.45 \pm 0.08 ^b	2.09 \pm 0.04 ^c	1.81 \pm 0.02 ^c	0.09 \pm 0.01 ^c	0.18 \pm 0.01 ^b	0.30 \pm 0.01 ^c
B, B, B80% ^{*c, a, b}	Total	3.17 \pm 0.18 ^a	2.13 \pm 0.17 ^a	1.50 \pm 0.15 ^{ab}	0.10 \pm 0.02 ^a	0.22 \pm 0.03 ^a	0.28 \pm 0.01 ^a
	Litter	3.80 \pm 0.54 ^a	2.12 \pm 0.26 ^a	1.28 \pm 0.14 ^a	0.06 \pm 0.02 ^a	0.14 \pm 0.03 ^{ab}	0.14 \pm 0.01 ^b
	Soil	2.84 \pm 0.03 ^a	2.22 \pm 0.09 ^a	1.79 \pm 0.15 ^b	0.04 \pm 0.01 ^a	0.09 \pm 0.01 ^b	0.14 \pm 0.01 ^b
C source		0.884	0.022	< 0.001	< 0.001	< 0.001	< 0.001
Moisture		< 0.001	< 0.001	0.037	< 0.001	< 0.001	< 0.001
Source \times Moisture		0.898	0.439	0.458	0.002	0.012	0.002

Respiration was sampled after 5 hours of incubation period. Soil moisture contents were 30, 60 and 80% MWHC. Between carbon source, within a moisture content, different letters (i.e., a, b, c) note significant differences (< 0.05). p-values at the base of table note the significance level of main effects and interaction shown at base of table. *Different letters (i.e. A, A, A) note the significant differences (< 0.05) for pairwise comparison of Q_{10} at 10, 20 and 30 °C (uppercase, left side), and dRs/dT at 10, 20 and 30 °C (lowercase, right side) respectively, between moisture contents.**Values include only 2 reps; one was excluded as an outlier due to unrealistic

Carbon source was not significant for Q_{10} values at 10 °C but was significant at 20 and 30 °C. There were significant differences between moisture contents, but no interaction between carbon source and moisture content was found. For dR_s/dT however, carbon source and moisture content were significant factors across all temperatures and significant interaction was observed. Pairwise comparison highlighted significant differences between Q_{10} at 30 and 60% MWHC across all temperatures, whilst dR_s/dT showed significant differences between 60 and 80% MWHC across all temperatures (Table 3.4).

3.5 Discussion

Temperature response of Litter and SOM decomposition.

Our developed protocol was very effective at separating temperature response of respiration from the two carbon pools in soil with litter and SOM displaying very different responses in respiration with increasing temperature. Respiration rates from litter had a very clear T_{opt} , peaking at ~40 °C, whilst respiration rates from SOM generally had much more of an Arrhenius response to increasing temperature. We hypothesise that decomposition of soil derived carbon (SOM-Rs) was more dependent on desorption of SOM from mineral surfaces and diffusion to microbes (governed by chemical processes) rather than enzyme interactions, resulting in an Arrhenius-like rate response. In contrast, respiration from added litter (Lit-Rs) was less limited by desorption and diffusion and more driven by enzymatic processes with a clear temperature optima (c.f. Schipper et al. 2019).

For temperature sensitivity, in all cases, Lit-Rs parameters T_{inf} and T_{opt} were less than Tot-Rs and more curvature (large negative c) was observed. In cases where SOM-Rs did have some slight curvature towards a T_{opt} (ΔC_p^\ddagger less than 0), the Lit-Rs was less than SOM-Rs for the same parameters. As a consequence of the differences in T_{inf} , T_{opt} and ΔC_p^\ddagger , differences were also observed in temperature sensitivity (Q_{10} or dRs/dT). Q_{10} was highest for SOM-Rs at 20 and 30 °C and in all cases Q_{10} declined with temperature increases, as is frequently reported (Conant et al., 2011; Schipper et al., 2014; Wang et al., 2019). dRs/dT increased with increasing temperature for SOM-Rs and Tot-Rs but peaked at 20 °C for Lit-Rs. Using similar approaches as here, Schipper et al. (2019) compared temperature response of irrigated and non-irrigated soils and found that irrigated soils had lower available carbon (lower CO_2 production) and the resulting temperature sensitivity of the irrigated soil was higher (higher Q_{10} , lower dRs/dT) than the non-irrigated. They hypothesised that differences in the temperature response of soil respiration are due to changes in substrate availability where relative substrate availability was assessed by CO_2 production at 10, 20 and 30 °C. Our results suggest that SOM decomposition has a higher relative temperature sensitivity (higher Q_{10}) at 20 and 30 °C than litter and a lower total CO_2 production, giving support to this hypothesis. More research using a wide range of soil and different carbon substrates is needed to determine the generality of the hypothesis that respiration of different pools of carbon in soils is dependent on the variable importance of physical chemistry processes (Arrhenius dominated) and enzymatic processes (described by MMRT).

This hypothesis is supported by a majority of studies that have found the stable carbon pool, or SOM pool, to be more temperature sensitive than labile carbon (Conant et al.,

2011; von Lützow & Kögel-Knabner, 2009; Wang et al., 2019). In contrast to this, we found that at lower temperatures Q_{10} for Lit-Rs can be slightly larger than SOM-Rs in some cases. As suggested by Conant et al. (2011) temperature sensitivity of soil respiration is likely a result of three temperature sensitive processes, the depolymerisation of carbon, the rate of enzyme production, and substrate availability. Our results suggest that at lower temperatures, the depolymerisation of carbon, or the rate of enzyme production may have a greater influence than substrate availability. Increased testing and method improvements for analyses with lower CO_2 production will help with accuracy at the lower temperature range.

Incubation time and moisture content effects.

While general patterns of Q_{10} and dRs/dT were mostly the same after 5 or 20 h of incubation, the size of differences was dependant on incubation time. Future comparisons should use a common incubation period (5 h) as it may be some microbial adaptation occurs, even within 20 h (Bradford, 2013). It is also possible that colonisation of added litter may alter microbial communities and their temperature responses, but our focus was to obtain immediate temperature responses once litter enters the soil rather than focus on longer term outcomes which might include adaptation or colonisation and successional processes. We also recommend the addition of a steady baseline of known CO_2 at the minimum analysis threshold of the OA-ICOS (~180 ppm). Adding a baseline will allow any CO_2 produced during a 5 hour incubation to be above the threshold for successful $\delta^{13}C$ analysis, even at low temperatures. This improvement will reduce any model fitting errors, associated with the reduction of data points due to low CO_2 production, and remove the need for extended incubation periods.

Moisture content had a pronounced effect on the temperature response of both Lit-Rs and SOM-Rs. Generally, moderate soil moisture contents (60% MWHC) are considered optimal for allowing oxygen (O_2) penetration into soil pores and diffusion to microbes while also ensuring sufficient water films for the supply of substrate to microbes (Sierra et al., 2015). Extreme dry conditions (30% MWHC) resulted in lower CO_2 production overall, most likely due to insufficient substrate supply, and smaller differences in the T_{inf} , T_{opt} , and ΔC_p^\ddagger between carbon sources. The decrease in substrate diffusion at 30% MWHC may have contributed to the lower T_{inf} and T_{opt} observed as a reduction in diffusion (Arrhenius-like response) would have magnified the relative importance of enzyme catalysis resulting in more curvature (lower ΔC_p^\ddagger) being exhibited in these cases. However, the decreasing ability to observe significant differences in the temperature-dependent parameters due to increased variability caused by moisture limitations may also have obscured any true temperature responses. Additionally, lower CO_2 concentrations reduced the number of successful analyses, reducing the number of data points, and causing more uncertainty in fitting the respiration model. This inability to analyse lower CO_2 concentration again could be resolved with continuing methodological improvements, as outlined above.

In extreme wet conditions (80% MWHC), CO_2 production was also reduced, likely due to reduced O_2 diffusion (Sierra et al., 2015). For example, for one replicate, respiration data became highly inconsistent resulting in unrealistic derived parameters (negative T_{opt} and T_{inf}). A reduction in not only O_2 diffusion but also CO_2 diffusion into the headspace could create more variability between each sample as water content varied slightly between tubes (Sierra et al., 2015). Also, at 80% MWHC, soils became difficult to manage which could lead to human error during the weighing process.

While for all moisture contents, Lit-Rs overall remained less temperature sensitive than SOM-Rs and Tot-Rs, future comparisons should use a standard, practical moisture content, such as 60% MWHC.

Method development

Using soil with added ^{13}C -labelled plant litter, incubated over short time intervals (5-29 hours), and at a wide range of temperatures (30 temperatures between ~ 2 and 50°C), we were able to separate the immediate temperature response of two carbon pools. A large number of individual data points allowed for robust fitting of the MMRT model (Robinson et al., 2017) or potentially other models, while a short incubation time reduced the potential effects of microbial adaptation that may occur with longer incubation periods (Bradford, 2013). While we used ^{13}C enriched litter as a secondary carbon pool to background SOM, other approaches where ^{13}C is incorporated into soil could be used to examine the responses of other carbon pools. As shown by Fierer et al. (2005), various enriched carbon compounds, ranging in complexity, could be added to soil to observe effects of carbon quality on the temperature response of soil respiration. The use of more homogeneously labelled carbon sources may also allow for determination of any priming effects on soil respiration due to the addition of carbon compounds (Kuzyakov, 2010). To account for more complexity, enrichment through root growth/root exudates could be used to examine the temperature response of more natural carbon pools. Additionally, shifts in C3-C4 plants that naturally label SOM over time may provide enough difference in soil $\delta^{13}\text{C}_{\text{VPDB}}$ for analysis of long-term carbon pool dynamics. Furthermore, although here we attributed lower respiration rates to lower availability of carbon, there could be other factors such as metabolism efficiency which may be worth investigation.

The main limitation of the method currently is the insufficient production of CO₂ concentrations from some soil samples (e.g. at low temperatures), which precluded analysis on the OA-ICOS and may limit the methodology to carbon-rich or altered (carbon added) systems. This limitation may be addressed by increasing the incubation period to allow more CO₂ production, but potential microbial growth or adaptation would need to be accounted for, we therefore recommend the addition of a CO₂ baseline at ~180 ppm, as described above.

3.6 Conclusions

Our preliminary experiments demonstrated that respiration from litter decomposition had lower T_{opt} and T_{inf} and was less temperature sensitive (Q_{10}) than SOM decomposition. We suggested this result was due to differences in carbon availability within each pool, particularly between 20 and 30 °C. However, the time of incubation and moisture content does alter temperature response and should be kept constant for future analyses. We have demonstrated a robust protocol to analyse temperature response of two distinct carbon pools, Lit-Rs and SOM-Rs, from soil. The method addresses inconsistencies with current analysis norms, allowing rapid analysis that minimises microbial adaptation and including 30 discrete incubation temperatures to increase the robustness of model fits. Methodological improvements such as including a known CO₂ baseline at the analysis threshold of the OA-ICOS, (~180 ppm), should increase the accuracy when analysing for ¹³C content at lower CO₂ concentrations. Improved ¹³C analysis will allow for future determination of temperature responses from numerous, diverse carbon pools using, added labelled carbon compounds, photosynthetic ¹³C enrichment of soil, or by utilising natural ¹³C/¹⁴C shifts.

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Conflict of Interest

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Chapter 4: Respiration derived from new photosynthate carbon and soil organic carbon have the same temperature response in irrigated and dryland soils.

Jasmine M. Robinson^{a*}, Carmen R. Carmona^{bc}, Samuel R. McNally^c, Michael H. Beare^c, Louis A. Schipper^a.

^aThe University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand

^bDepartment of Soil and Physical Sciences, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand.

^cPlant & Food Research, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140

The contributions of authors were (see co-authorship form at back of thesis):

Jasmine Robinson, Louis Schipper and Mike Beare decided on experimental design. Carmen Carmona with help from Sam McNally, contributed enriched soils and litter for analysis. Jasmine Robinson was responsible for running experiments, analysing data, statistical analyses and writing the manuscript. Louis Schipper was the primary reviewer for this manuscript.

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4.1 Abstract

Assessing the vulnerability of soil carbon to decomposition under different management such as irrigation is essential for accurate modelling of changes in carbon stocks and informing ideal management practices. However, how different pools of carbon, including new carbon inputs, respond to increasing temperature, particularly under differing conditions, is understudied. Using a recently developed protocol, we measured the temperature response of respiration derived from ^{13}C -labelled, new photosynthate carbon inputs and bulk soil organic carbon (SOC) from soil from seasonal dryland and irrigated treatments. We also measured respiration of ^{13}C -labelled root and shoot litter mixed with unlabelled soil. Respiration from new photosynthate carbon had the same Arrhenius-like temperature response as respiration from soil organic carbon under both treatments. In contrast, added root and shoot litter was well described by macromolecular rate theory (MMRT) with a temperature inflection point (T_{inf} ; dryland) of about 22 and 18 °C, respectively. We attribute the similarity of respiration of new photosynthate carbon inputs and SOC to the new carbon being rapidly incorporated into soil, either due to the proximity of root inputs to microbially active zones adjacent to soil surfaces where carbon can be stabilised or by having a similar degradability as SOC upon entry. Either rationalisation suggests that carbon inputs to soil from live roots are more stable than bulk deposition of root or shoot litter. Dryland and irrigated treatments had differing temperature responses, with irrigation having a much lower temperature sensitivity (Q_{10}) and a higher T_{opt} and T_{inf} . These differences were attributed to an increase in short-term turnover of carbon under irrigation.

4.2 Introduction

Soil organic carbon (SOC) storage in the biosphere is a balance mostly between photosynthetic inputs (litter and root exudates) and carbon dioxide (CO₂) outputs via microbial decomposition (von Lützow & Kögel-Knabner, 2009). As soils may contain up to 81% of global, actively cycling, organic carbon stocks (von Lützow & Kögel-Knabner, 2009), management processes that alter the balance and release even a small percentage of this carbon will have a large, potentially negative impact on the biosphere as a whole and contribute to atmospheric concentrations of CO₂ (Bradford et al., 2016; Lal, 2004; Schmidt et al., 2011). We rely on soils for global food production and so understanding how management processes such as irrigation or cropping affect microbial decomposition of soil carbon is vital for maintaining carbon stocks whilst still allowing sufficient production for a growing population (Whitehead et al., 2018). As soil carbon storage is increasingly recognised as the consequence of a complex interacting system of biological, physical and chemical processes, it becomes reasonable to assume that soil management and temperature increases may not affect all forms of carbon in soil equally (Conant et al., 2011; Davidson & Janssens, 2006). Generally, there is considered to be at least two conceptual pools of carbon in soil, an active/labile pool available for microbial decomposition, and a protected/stable pool that is somewhat (either chemically or physically) biologically unavailable (Lehmann & Kleber, 2015). Determining how these carbon pools change and stabilise with regards to soil management and future climate variability will make it easier to reliably predict and manage future environmental impacts (Bradford et al., 2016; Davidson & Janssens, 2006).

Irrigation is one key management practice that is used to increase food production and alters both carbon inputs and outputs from the soil, and thus carbon storage (Trost et al., 2013). In general, irrigation increases above-ground biomass, but in some cases can reduce root biomass while also increasing microbial decomposition of soil carbon (Scott et al., 2012; Trost et al., 2013). However, how irrigation might alter carbon stabilisation within the soil remains unclear. In parts of New Zealand, despite a reliance on summer irrigation and a substantial increase in usage, New Zealand specific data on carbon storage under irrigation is limited (Mudge et al., 2017; Scott et al., 2012). Whitehead et al. (2018) reviewed studies investigating the effects of management practices, including irrigation, on soil carbon stocks. Of the New Zealand based studies reviewed, losses (Condon et al., 2014; Kelliher et al., 2012; Mudge et al., 2017; Schipper et al., 2013), gains (Laubach & Hunt, 2018), and no change (Kelliher et al., 2015), in total carbon, have all been reported highlighting a lack of certainty on how total carbon changes under irrigation. And while studies on total carbon may provide an insight on changes to soil carbon stocks, how carbon transfers between different labile and stable carbon pools within a soil profile and is stabilised or destabilised due to changes in management is difficult to determine (Jackson et al., 2017).

There have been few studies of the comparative flows of carbon in irrigated and non-irrigated soils. Carmona et al. (2020b) compared mechanisms of carbon storage and stabilisation in soil under typical New Zealand irrigation and dryland conditions, using enriched CO₂ (¹³CO₂) pulse-labelling, traced carbon allocation of ryegrass/clover pasture in a mesocosm experiment. Soils were labelled under irrigation and dryland conditions for three months, and then changes in carbon allocation were measured using destructive sampling at five intervals over 349 days, under the same moisture inputs. They found that along with expected results of increased above-ground

biomass and reduced root-biomass under irrigation, total new carbon inputs were not significantly different under irrigation compared to dryland treatments. However, although irrigation did not appear to increase total carbon inputs, there was an increase in the amount of new photosynthate material present in the fine particulate organic matter (POM) fraction (53–250 μm) and the clay fraction ($< 5 \mu\text{m}$) compared to dryland treatments. They concluded that more new material in the fine POM clay fractions was indicative of increased root turnover, aggregation, and potential stabilisation of carbon under irrigated pasture due to increased biological activity with increased water availability (Carmona et al., 2020b).

Increased biological activity or increased respiration is considered a major reason for changes to carbon storage under irrigation (Carmona et al., 2020b; Mudge et al., 2017; Scott et al., 2012). However, these studies have generally not measured how respiration responds to climatic variables such as temperature and how management practices influence these. Recently, Schipper et al. (2019) studied the temperature sensitivity of soil respiration from 32 paired, irrigated and non-irrigated sites throughout New Zealand. They used macromolecular rate theory (MMRT) to describe and compare the temperature response of these irrigated and dryland soils. MMRT allows calculation of the T_{inf} - the temperature at which the change in respiration rate is maximal, and T_{opt} - the temperature where respiration rate is maximal (Alster et al., 2020; Schipper et al., 2014). Two metrics of temperature sensitivity (absolute and relative) were also calculated, including the first derivative (absolute temperature sensitivity: rate of change of the respiration curve) and Q_{10} (relative temperature sensitivity: the ratio of rates of respiration measured 10 °C apart). Schipper et al. (2019) measured a higher T_{opt} , T_{inf} and lower respiration rates under irrigation than non-irrigated sites which they attributed to lower carbon availability for microbial decomposition. Additionally, they

calculated a higher Q_{10} under irrigation that also indicated lower carbon availability as it is generally understood that less labile substrates typically have higher relative temperature sensitivities (Conant et al., 2011; Larionova et al., 2007; von Lützow & Kögel-Knabner, 2009).

Although the consensus of less labile substrates having higher relative temperature sensitivities (Q_{10}) exists, accurately measuring the temperature sensitivity of multiple carbon pools in a single soil is still relatively difficult and inconsistent. Robinson et al. (2020) described a new method for measuring the temperature sensitivity of soil respiration from two pools of carbon within one soil using litter labelled with enriched carbon (^{13}C). Using a temperature gradient block, they incubated soils mixed with a ^{13}C enriched plant litter and analysed the resulting CO_2 produced for ^{13}C to allow separation of total respiration into soil-sourced and litter-sourced respiration. While this method was developed using ^{13}C labelled litter, the general approach is also applicable to other ^{13}C labelled substrates. With this method and subsequent testing, they found that enriched litter additions to the soil had a significantly lower relative temperature sensitivity (Q_{10}) than the soil organic matter (Robinson et al., 2020). This difference was attributed to the very high availability of the added litter to microorganisms compared to the soil organic matter, supporting conclusions by Schipper et al. (2019), who used a similar incubation method. Using added litter, harvested from above-ground material, as a second carbon pool, however, is likely not comparable to natural inputs of belowground carbon from roots, as differences in the retention and decomposition of root-derived carbon compared to residue/litter carbon has been noted in the literature (Córdova et al., 2018; Fulton-Smith & Cotrufo, 2019; Kong & Six, 2010). Additionally, it has been suggested that different pathways (in vivo: carbon passes through a microbe, or direct sorption: does not pass through a

microbe) of carbon addition to soil may select opposing substrate properties for incorporation and allocate carbon differently within soil fractions (Sokol et al., 2019b).

Our main objective was to compare the temperature sensitivity of soil respiration from soil organic carbon and new photosynthate carbon inputs (enriched carbon sourced from root exudates and root/shoot derived litter) deposited under irrigated and dryland conditions below a pasture sward. We obtained bulk soil samples and litter samples from the experiment described above by Carmona et al. (2020b). We incubated these ^{13}C enriched soils, then analysed the resulting CO_2 and its isotopic signature using the method described by Robinson et al. (2020) to determine the temperature sensitivity from both the newly added photosynthate carbon and old carbon sources. Due to the increase in new photosynthate carbon in the $< 5 \mu\text{m}$ POM fraction in the irrigated soil (Carmona et al., 2020b), and a reduction of available substrate postulated by Schipper et al. (2019), we hypothesised that there would be less biologically available carbon under irrigation as it had previously been more stabilised in the fine POM fraction and clay fractions. If this hypothesis were correct, we expected to observe lower total respiration rates and changes in temperature sensitivity (higher Q_{10} and lower dR_s/dT) under irrigation compared to dryland treatment.

Differences in the temperature response of respiration between new photosynthate inputs and pre-existing SOC from before the imposition of treatments were harder to predict. As described in Robinson et al. (2020), highly available litter additions had a much lower T_{inf} and T_{opt} than SOC. The new photosynthate material in the samples from Carmona et al. (2020b) was considered not as available as litter since it has entered the soil through root turnover and exudation and possibly more opportunity

for stabilisation than bulk added litter. We predicted new photosynthate carbon would have a lower temperature sensitivity than SOC; however, not to the same extent as the litter additions observed in Robinson et al. (2020).

4.3 Methodology

Soil sampling and labelling

This experiment used soils from a study by Carmona et al. (2020b), where full experimental details can be found. Briefly, they collected a Lismore stony silt loam soil (Udic Ustochrept, 4 g C kg⁻¹ soil, 0-15 cm depth) from an established dryland Lucerne pasture (volumetric water content (VWC) at field capacity: 39%, and at permanent wilting point: 13%), from Ashley Dene Research and Development farm (Lincoln, Canterbury, NZ). After sieving to remove stones (6 mm), soil was repacked into 60 separate microcosms (~ 15 cm in diameter and 25 cm deep), with a similar stone content (50% gravimetric stone content) and soil fine earth bulk density (1.10 g cm⁻³) as *in situ* conditions. Over a five-month period, the microcosms were kept at a constant winter-spring moisture content (20 – 32% VWC) in an unheated greenhouse whilst a ryegrass-clover pasture was established. Soils were periodically fertilised with a solution of nitrogen (N) (15 g L⁻¹ urea solution, equivalent to 50 kg N ha⁻¹) and P-K-S (19 kg P ha⁻¹, 159 kg K ha⁻¹ and 16 kg S ha⁻¹) to sustain adequate growth (Carmona et al., 2020b).

One month before they labelled soil with ¹³C-CO₂, each microcosm was randomly assigned one of two treatments that represented either a typical New Zealand dryland (maintained at 13 – 28% VMC) or an irrigated (maintained at 25 – 33% VWC) pasture during summer. Microcosms were weighed weekly and watered when required. For

labelling, 48 microcosms (24 from each treatment) were placed into a large constructed chamber (sealed transparent acrylic, 2 m length \times 1.2 m width \times 1 m height) in a randomised block design ($n = 4$), where environmental conditions could be monitored constantly, and labelling could occur (Carmona et al., 2020b). Twelve microcosms, six from each treatment, were kept aside in a greenhouse under similar conditions as a natural abundance control.

Over a three-month period, the chamber received daily additions of evolved $^{13}\text{CO}_2$ (equivalent to 0.5 g $\text{Na}_2^{13}\text{CO}_3$) delivered from a gasbag using a precision air pump. Total CO_2 , $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ concentration were continuously monitored via a data logger (Campbell Scientific CR1000, USA) and a cavity ring down spectrometer (Picarro G2210-i, Santa Clara, CA). Background CO_2 concentration within the chamber was maintained at ~ 400 ppm; if this exceeded 700 ppm before morning labelling, the chamber was manually opened to remove CO_2 .

After the labelling period, a chase period where all microcosms were kept at the same moisture contents (following standard seasonal rainfall) was initiated. At five time periods (Time 1 = 1 day, Time 2 = 12 days, Time 3 = 125 days, Time 4 = 237 days, Time 5 = 349 days) after labelling, destructive sampling was completed between 0 – 15 cm and 15 - 25 cm depths for various soil properties including ^{13}C ‰, total carbon and total nitrogen of rhizosphere, non-rhizosphere and bulk soil. Remaining soil from each microcosm was air-dried, sieved to 2 mm, and kept at 4 °C. Root and shoot samples were also kept.

For the current study, four replicate microcosms from each treatment at three sampling times were selected that had sufficient residual bulk soil (Time 1, Time 3 and Time 5) for determining temperature response (Figure 4.1).

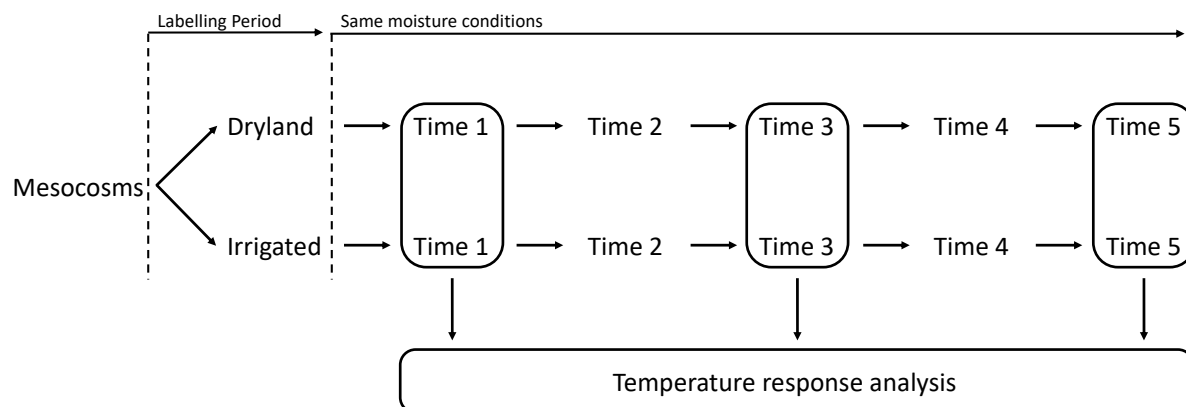


Figure 4.1: Basic timeline of the three-month ^{13}C labelling process, and subsequent chase period. Sample times 1 (1 day after labelling), 3 (125 days after labelling) and 5 (349 days after labelling) were sub-sampled for temperature response analysis.

Soil was also collected from three control (non-labelled) microcosms from each treatment from Time 1 and combined treatments from Time 5 to use a reference of normal CO_2 production (Appendix 2). In total, there were 33 soils (4 labelled replicates \times 2 treatments, at 3 sampling times = 24 total) and nine control replicates (3 Dryland and 3 Irrigated samples from T1, 2 Dryland samples 1 Irrigated sample from T5). In addition to this experiment, combined root material from two of the four selected replicates, and a combined litter sample from each treatment, from Time 1 were ground up for use as artificial carbon additions to control soil.

Root, shoot, and soil ^{13}C ‰, total carbon and root mass values are given in Table 4.1.

Table 4.1: Root ^{13}C ‰ values and bulk soil total carbon for sampled mesocosms, values Carmona (2020).

	Mesocosm I.D.	Enrichment (^{13}C ‰)	Total carbon (%)
Time 1	10	109.72	3.25
	25	103.40	3.10
	30	106.46	3.12
	43	105.76	3.12
	Root	104.58*	n/a**
	Shoot	900*	n/a**
	20	115.53	3.18
	29	114.93	3.21
	32	94.73	3.20
	37	136.75	3.17
	Root	115.74*	n/a**
	Shoot	900*	n/a**
Time 3	4	68.12	3.17
	18	59.67	3.16
	31	109.96	3.15
	39	102.98	3.10
	11	172.76	3.09
	16	116.73	3.15
	34	164.42	3.08
	38	100.23	3.11
Time 5	1	9.01	3.16
	14	2.13	3.20
	35	5.98	3.20
	47	14.16	3.22
	2	11.42	3.13
	15	30.61	3.16
	28	13.55	3.16
	45	28.39	3.15

*Samples are composites of multiple mesocosms.

** Values not determined

Temperature sensitivity analysis.

Soils were wet up to 60% maximum water holding capacity (MWHC) as calculated in Harding and Ross (1964), equivalent to ~28% VWC, seven days prior to incubation. Labelled soils were weighed (4 g) into 30 Hungate tubes (38 mL) and incubated for 5 hours between ~2 and 50 °C. For the control + labelled root/shoot litter experiment, soil was weighed out (4 g) into tubes with ~0.001 g of root or shoot litter, shaken to mix, then incubated. After incubation, the Hungate tubes were immediately placed into ice to halt further respiration and stored at -20 °C overnight for subsequent ^{13}C -CO₂ analysis.

For ^{13}C -CO₂ analysis, headspace gas samples were run through a modified Los Gatos OA-ICOS CO₂ analyser as described in Robinson et al. (2020). A selection of five international (NSB-1: 1.95 ‰, sigma: -14.18 ‰, BDH: -24.95 ‰, as reported in Beinlich et al. (2017)) and internal standards (range of bicarbonate solutions from ~30 ‰ to 250 ‰, standardised using the international reference carbonate standards) were run alongside samples to allow reporting of values to $\delta^{13}\text{C}_{\text{VPDB}}$. Replication of standards reported an error of ~0.05‰.

To improve analysis of low CO₂ concentrations in headspace samples in comparison to Robinson et al. (2020), a baseline carrier gas (0.15 % CO₂, 1% Ar, and 21% O₂, balance N₂, mixed with a scrubbed N₂ gas) at ~180 ppm CO₂ was used to transfer headspace gas samples so any added CO₂ would be above analysis threshold of 160 ppm. To reduce any effects a range of peak areas may have on $\delta^{13}\text{C}$ analysis, it is suggested that the standards used should also include a range of concentrations to allow for correction if necessary. Using peak area to calculate $\delta^{13}\text{C}$ also meant total

CO₂ could be quantified from the OA-ICOS rather than using subsamples through an infra-red gas analyser (IRGA). Three replicates of five CO₂ gas concentrations (1% CO₂ in Nitrogen) were run on the OA-ICOS encompassing the predicted CO₂ concentrations.

Data analysis

By using the total CO₂ produced during incubation, new photosynthate carbon (¹³C labelled) and soil-derived CO₂ were calculated using a 2-pool mixing model (Equation 4.1).

$$f = \frac{(C_S - C_C)}{(C_R - C_C)} \quad \text{Equation 4.1}$$

Where C_S, C_C, and C_R are the δ¹³C_{VPDB} values of the soil, the total respired CO₂, and the enriched root values (representative of the new photosynthate material), respectively.

Partitioning CO₂ using δ¹³C_{VPDB} resulted in three individual temperature-respiration curves (Figure 4.2):

- a) combined soil + new photosynthate (Tot-Rs)
- b) new photosynthate carbon-derived (NPC-Rs)
- c) soil organic carbon-derived (SOC-Rs) respiration rate

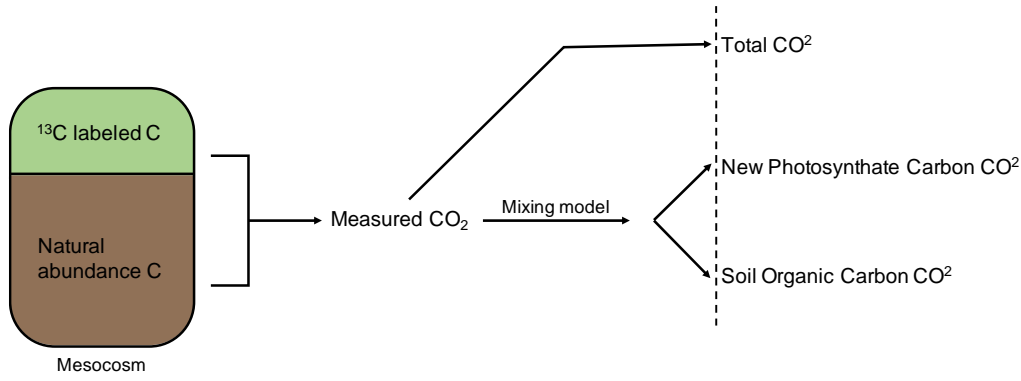


Figure 4.2: Representation of the origin of the three compared CO_2 curves.

The temperature responses of the resulting separate respiration rates (R_s) were fitted using MMRT (Equation 2) (Hobbs et al., 2013; Schipper et al., 2019). The temperature optimum (T_{opt}) and inflection point (T_{inf}) were calculated using Equation 4.3 and Equation 4.4. Two metrics of temperature sensitivity, the first derivative and Q_{10} , were also calculated (Sierra, 2012).

$$\ln(R_s) = \ln \left[\frac{k_B T}{h} \right] - \frac{\Delta H_{T_0}^\ddagger + \Delta C_p^\ddagger (T - T_0)}{RT} + \frac{\Delta S_{T_0}^\ddagger + \Delta C_p^\ddagger (\ln(T) - \ln(T_0))}{R} \quad \text{Equation 4.2}$$

$$T_{\text{opt}} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger - R} \quad \text{Equation 4.3}$$

$$T_{\text{inf}} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_p^\ddagger T_0}{-\Delta C_p^\ddagger \pm \sqrt{-\Delta C_p^\ddagger R}} \quad \text{Equation 4.4}$$

Where ΔC_p^\ddagger ($\text{J mol}^{-1} \text{ K}^{-1}$) is the change in heat capacity between the enzyme-substrate complex and the enzyme bound to the transition state, k_B is Boltzmann's constant, and h is Planck's constant. In the case of soil respiration, the ΔC_p^\ddagger is the approximate ensemble mean ΔC_p^\ddagger of all the enzymes involved in respiration (Schipper et al., 2014). $\Delta H_{T_0}^\ddagger$ is the change in enthalpy (J mol^{-1}), and $\Delta S_{T_0}^\ddagger$ ($\text{J mol}^{-1} \text{ K}^{-1}$) is the change in entropy between the enzyme-substrate complex and the enzyme bound to the transition state at a reference temperature (T_0 , which we set to 298 K, 25 °C).

All peak calculations and data processing were performed using MATLAB R2017b (The MathWorks Inc., Natick, MA, USA). The number of replicates used, although four were sampled, varied between 4-2 due to sampling and measurement errors. Replicates were removed based on high data variability (i.e. reps removed if; after bootstrapping 2000 times, the number of MMRT fits that laid within the 95% confidence bounds of the average fit was less than 20, if negative T_{inf} and T_{opt} values were calculated, or if respiration rates were inexplicably low).

Statistical analyses were performed using GenStat (64-bit Release 20.1). To take into account the split-plot design (NPC and SOC result from the same respiration measurement) and uneven replicates, a Two-way ANOVA was used to test for differences in carbon source for each sampling time individually. A Two-way ANOVA using regression was used to investigate the joint effects of time and treatment on either the mean source value (removing the influence of carbon source) or the difference in NPC – SOC. The treatment variable was added after the sampling time variable, so any sampling time effects do not confound any significant treatment

effects. Further analysis compared the similarity of predicted values from the regression model.

It is important to note that there are limitations in using isotopic values of root material to represent new carbon inputs over time (Figure 4.3). In this study, the isotopic value of the root material at Time 1 was assumed an appropriate representation of available new carbon inputs to the soil during the labelling period (prior to Time 1). However, for Time 3 and 5, as labelled carbon was redistributed throughout the profile, and root $\delta^{13}\text{C}$ values are diluted by new, natural abundance carbon, using the measured root $\delta^{13}\text{C}$ value from these Time 1 may not be representative of the labelled carbon in the soil system.

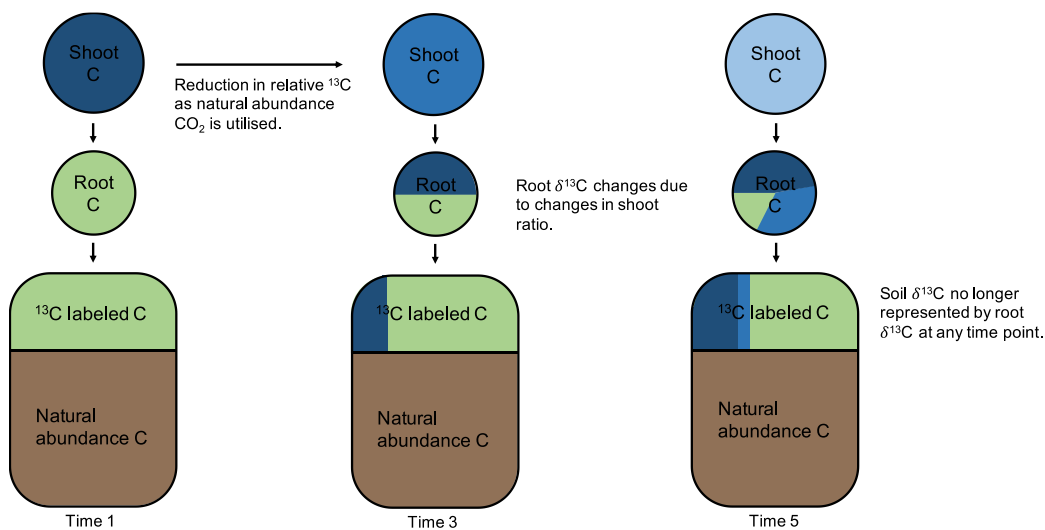


Figure 4.3: Representation of how the isotopic value of shoots, root and soil carbon changes over time and to different extents. At later time points (Time 3 and 5), the $\delta^{13}\text{C}$ root carbon is no longer representative of new carbon inputs to soil.

We suggest that changing this value would not affect the shape of the respiration curve produced, but rather the proportion of respiration from the labelled source may be consistently underestimated. We focus instead on MMRT parameters rather than Q_{10} values when comparing sampling times, as these rely on the curve shape rather than the total amount of CO_2 from each source.

4.4 Results

We compared resulting temperature response curves for three CO_2 sources (combined soil + new photosynthate, new photosynthate carbon-derived (NPC-Rs), and soil organic carbon-derived (SOC-Rs) respiration rate) between irrigated and dryland treatments, and between three soil sampling times (T1 = 1 day, T3 = 125 days and T5 = 349 days after labelling ended). Comparisons in this section and the discussion will be focused on differences and similarities between the two distinct pools in soil SOM-Rs and NPC-Rs.

In this section, we also focus on comparisons of MMRT parameters rather than measures of temperature sensitivity. These parameters are less likely to be affected by the potential misrepresentation of new photosynthate carbon in soil, at later time points during the chase period (Figure 4.3).

Results from incubation of ^{13}C labelled soils

Respiration rates generally increased with temperature increases as expected and showed some curvature to an optimum at higher temperatures (Figure 4.4).

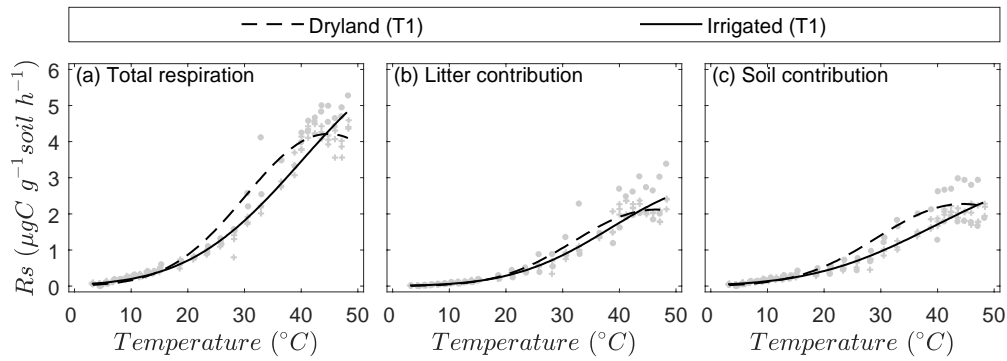


Figure 4.4: Respiration rates (R_s) from a 5-hour incubation of a ^{13}C labelled Limore soil (60% MWHC), sampled 1 day (Time 1) after the end of labelling. Total respiration (a) was partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c) using a mixing model. All were fitted using the MMRT model. ^{13}C label was applied under either dryland and irrigated treatments, symbols indicate 4 replications of data for dryland (•) and irrigated treatments (+), different lines represent average response for different these treatments.

When comparing respiration rates of NPC-Rs and SOC-Rs at 10, 20 and 30 $^{\circ}\text{C}$ (Table 4.2), SOC-Rs was mostly higher, but only sometimes significantly. Overall, irrigated respiration rates (Irr-Rs) were typically lower than the equivalent dryland respiration rates (Dry-Rs).

Table 4.2: Calculated MMRT parameters, respiration rates, Q_{10} and dRs/dT (mean \pm std*), derived from a ^{13}C enriched Limore soil, sampled 1 day (Time 1), 125 days (Time 3) and 349 days (Time 5) after labelling.

	Treatment	C	T_{opt}	T_{inf}	ΔC_p^{\ddagger}	Rs at 10 °C	Rs at 20 °C	Rs at 30 °C	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dRs/dT at 10 °C	dRs/dT at 20 °C	dRs/dT at 30 °C
		source	(°C)	(°C)	(J mol ⁻¹ K ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)				(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)
Time 1	Dryland	NPC	48 \pm 4	33 \pm 2	-3962 \pm 997	0.05 \pm 0.01	0.32 \pm 0.12	1.06 \pm 0.44	6.42 \pm 1.34	3.28 \pm 0.18	1.85 \pm 0.14	0.011 \pm 0.003	0.049 \pm 0.022	0.094 \pm 0.038
		SOC	44 \pm 1	29 \pm 1	-3688 \pm 30	0.11 \pm 0.02	0.54 \pm 0.09	1.42 \pm 0.27	4.73 \pm 0.13	2.60 \pm 0.08	1.53 \pm 0.05	0.022 \pm 0.004	0.068 \pm 0.013	0.097 \pm 0.021
	Irrigated	NPC	58 \pm 2	39 \pm 2	-2502 \pm 147	0.06 \pm 0.01	0.29 \pm 0.04	0.86 \pm 0.06	4.68 \pm 0.48	3.02 \pm 0.25	2.05 \pm 0.14	0.011 \pm 0.002	0.037 \pm 0.003	0.077 \pm 0.005
		SOC	66 \pm 13	41 \pm 8	-1627 \pm 442	0.14 \pm 0.03	0.42 \pm 0.06	0.96 \pm 0.09	3.10 \pm 0.29	2.31 \pm 0.16	1.79 \pm 0.16	0.017 \pm 0.003	0.041 \pm 0.005	0.067 \pm 0.005
Time 3	Dryland	NPC	61 \pm 5	40 \pm 4	-2130 \pm 383	0.15 \pm 0.09	0.57 \pm 0.26	1.51 \pm 0.56	4.10 \pm 0.84	2.79 \pm 0.42	1.99 \pm 0.22	0.023 \pm 0.011	0.065 \pm 0.025	0.123 \pm 0.034
		SOC	55 \pm 4	32 \pm 1	-1805 \pm 464	0.22 \pm 0.02	0.63 \pm 0.17	1.30 \pm 0.48	2.80 \pm 0.47	2.04 \pm 0.19	1.55 \pm 0.04	0.027 \pm 0.008	0.055 \pm 0.024	0.076 \pm 0.036
	Irrigated	NPC	70 \pm 11	47 \pm 8	-1984 \pm 492	0.03 \pm 0.01	0.16 \pm 0.04	0.51 \pm 0.12	4.64 \pm 0.53	3.21 \pm 0.29	2.32 \pm 0.25	0.006 \pm 0.002	0.021 \pm 0.006	0.050 \pm 0.008
		SOC	66 \pm 6	42 \pm 3	-1809 \pm 351	0.17 \pm 0.05	0.62 \pm 0.11	1.65 \pm 0.23	3.70 \pm 0.44	2.65 \pm 0.17	1.98 \pm 0.03	0.025 \pm 0.005	0.070 \pm 0.010	0.135 \pm 0.017
Time 5	Dryland	NPC	68 \pm 6	45 \pm 3	-1978 \pm 494	0.11 \pm 0.08	0.45 \pm 0.10	1.35 \pm 0.23	4.46 \pm 0.98	3.07 \pm 0.43	2.21 \pm 0.16	0.017 \pm 0.005	0.057 \pm 0.010	0.128 \pm 0.025
		SOC	70 \pm 20	45 \pm 11	-1725 \pm 672	0.12 \pm 0.07	0.37 \pm 0.21	0.90 \pm 0.51	3.53 \pm 0.67	2.56 \pm 0.27	1.94 \pm 0.07	0.015 \pm 0.008	0.037 \pm 0.022	0.069 \pm 0.038
	Irrigated	NPC	71 \pm 3	47 \pm 4	-1748 \pm 113	0.08 \pm 0.03	0.32 \pm 0.06	0.93 \pm 0.02	4.11 \pm 0.72	2.95 \pm 0.44	2.21 \pm 0.28	0.012 \pm 0.003	0.039 \pm 0.002	0.086 \pm 0.011
		SOC	58 \pm 8	36 \pm 5	-1998 \pm 499	0.17 \pm 0.06	0.57 \pm 0.25	1.34 \pm 0.61	3.33 \pm 0.28	2.35 \pm 0.03	1.73 \pm 0.09	0.024 \pm 0.011	0.059 \pm 0.028	0.092 \pm 0.039

Respiration (Rs, μgC g⁻¹ h⁻¹) was sampled at the end of a 5 hour incubation period. Bold values indicate the significant differences (probability that predicted difference includes 0; < 0.05) sources, within a treatment.

*The number of replications varied due to sampling and measurement error, numbers were 3, 4, 3, 4, 2 respectively for the six different measurements downwards

After respiration rates were fitted with MMRT, parameters T_{opt} , T_{inf} , and ΔC_p^\ddagger were compared between carbon sources, treatments, and sampling times. For these fitted parameters, within a sampling time and treatment, NPC-Rs and SOC-Rs were not significantly different (Table 4.2) except for the two cases of ΔC_p^\ddagger (Time 1, Irrigated) and T_{inf} (Time 3, Dryland).

From the MMRT fits produced, measures of temperature sensitivity (Q_{10} and dRs/dT) were also calculated. Q_{10} declined with increasing temperature as expected and varied from 6.4 to 1.5, while dRs/dT increased with increasing temperature up to $\sim 30^\circ\text{C}$ and varied between 0.135 and 0.006 (Figure 4.5).

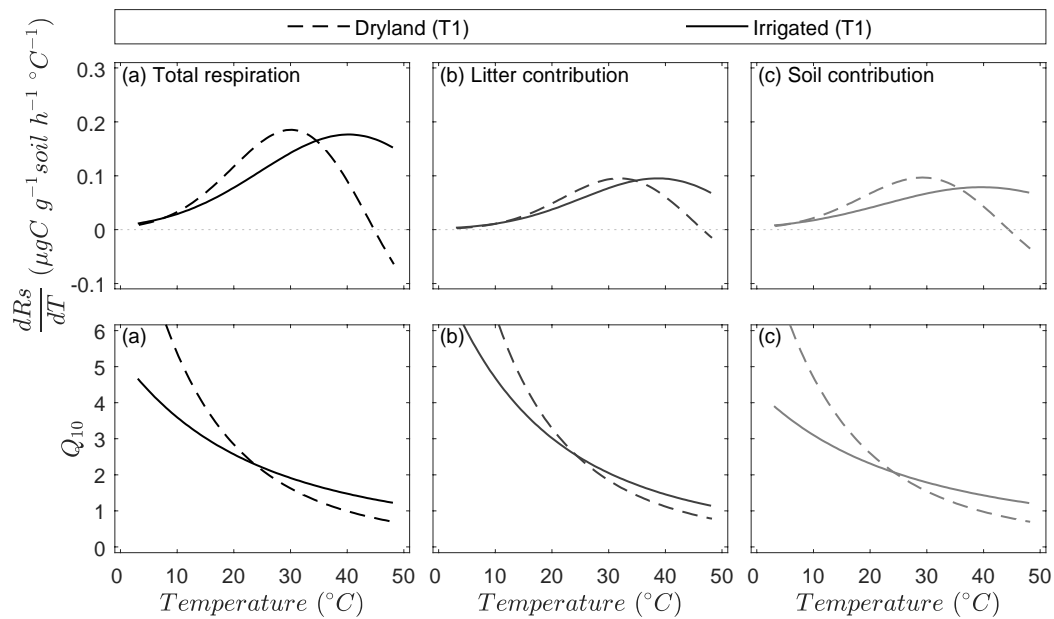


Figure 4.5: First derivative (dRs/dT) and Q_{10} calculated from MMRT model fits of respiration rates from a 5-hour incubation of a ^{13}C labelled Limore soil (60% MWHC), labelled under dryland or irrigated treatments, sampled 1 day after labelling ceased(T1). Total respiration has been partitioned into respiration from new photosynthate carbon and bulk soil organic carbon. Different lines represent average temperature sensitivity ($n = 4$) for different treatments.

When compared at 10, 20 and 30 °C, in every case, Q_{10} for NPC-Rs was almost always significantly higher than SOC-Rs, but inconsistently at Time 5 (Table 4.2). Similarly, dRs/dT was lower for NPC-Rs than SOC-Rs in most cases, but only sometimes significantly.

An accumulated analysis of variance was used to observe the influence of sampling time and treatment type on the difference between NPC and SOC (Table 4.3). A significant p-value in this table would imply that time or treatment has influenced values of NPC and SOC differently for that variable (i.e. the difference between the two values has changed). This same analysis was used to observe the influence of sampling time and treatment on the mean carbon source value (averaged value of NPC and SOC; Table 4.4). A significant p-value in this table would imply that time or treatment has influenced SOC and NPC values in general for that variable (i.e. both values have increased). The focus of differences throughout time will be on MMRT parameters and Q_{10} . Again, it is important to note that comparisons of respiration rate and dRs/dT over time may not fully represent temporal changes of the NPC and SOC pools in soil due to the potential inaccuracies following the use of root $\delta^{13}C$ as endpoints for partitioning.

Table 4.3: Significance level (p-value) from accumulated analysis of variance for the difference between NPC and SOC for calculated MMRT parameters, respiration rate and temperature sensitivity values. Significant values ($P < 0.05$) are shown in bold.

		T _{opt}	T _{inf}	ΔC _p [‡]	Rs at 10 °C	Rs at 20 °C	Rs at 30 °C	Q ₁₀ at 10 °C	Q ₁₀ at 20 °C	Q ₁₀ at 30 °C	dRs/dT at 10 °C	dRs/dT at 20 °C	dRs/dT at 30 °C
		(°C)	(°C)	(J mol ⁻¹ K ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)				(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)
NPC - SOC	Time	0.556	0.424	0.393	0.319	0.280	0.202	0.383	0.730	0.561	0.315	0.267	0.090
	Treatment	0.927	0.956	0.930	0.194	0.106	0.044	0.653	0.856	0.974	0.141	0.065	0.009
	Interaction	0.278	0.232	0.390	0.690	0.171	0.070	0.967	0.772	0.341	0.193	0.964	0.021

Table 4.4: Table 4: Significance level (p-value) from accumulated analysis of variance for the mean source value (averaged NPC and SOC value) of calculated MMRT parameters, respiration rate and temperature sensitivity values. Significant values ($P < 0.05$) are shown in bold.

	T _{opt}	T _{inf}	ΔC _p [‡]	Rs at 10 °C	Rs at 20 °C	Rs at 30 °C	Q ₁₀ at 10 °C	Q ₁₀ at 20 °C	Q ₁₀ at 30 °C	dRs/dT at 10 °C	dRs/dT at 20 °C	dRs/dT at 30 °C	
	(°C)	(°C)	(J mol ⁻¹ K ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)				(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	
Mean source	Time	0.010	0.010	<.001	0.014	0.074	0.250	0.020	0.614	0.017	0.040	0.455	0.247
	Treatment	0.019	0.013	0.001	0.198	0.032	0.026	0.058	0.862	0.007	0.051	0.014	0.095
	Interaction	0.040	0.027	<.001	0.012	0.066	0.276	0.002	0.01	0.014	0.032	0.137	0.633

For differences in NPC and SOC (Table 4.3), analysis of variance highlighted that sampling time and treatment type was not a significant influence for most measured parameters. This lack of significance suggests that any of the significant differences between measured NPC and SOC values highlighted previously in Table 4.2 (In bold), such as those for Q_{10} and respiration, can be attributed solely to differences between the carbon sources (Table 4.3).

For averaged values of NPC and SOC, analysis of variance showed that for almost all calculated parameters, either sampling time, treatment, or a combination of both variables, had a significant influence on mean source value (Table 4.4). This significance indicated that general parameter values of NPC and SOC were likely a result of the imposed treatment or sampling time, and further investigation was required (Table 4.4). Mean source values predicted from the regression model were used to explore further how treatments and sampling times were affecting predicted parameter values (Table 4.5).

Table 4.5: Predicted mean source MMRT parameters, respiration rate and temperature sensitivity values for dryland and irrigated treatments at three sampling times. Significant differences ($P < 0.05$) between dryland and irrigated treatments within a time (across) are bold. Significant differences ($P < 0.05$) between sampling times (down) within treatment are denoted by lower-case letters (i.e. a, b, ab).

	T_{opt} (°C)		T_{inf} (°C)		ΔC_p^\ddagger (J mol ⁻¹ K ⁻¹)	
	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
T1	46 ± 4^a	62 ± 3^a	31 ± 2^a	40 ± 2^a	-3825 ± 205^a	-2064 ± 178^a
T3	58 ± 4 ^b	68 ± 4 ^a	36 ± 2^a	45 ± 2^a	-1968 ± 205 ^b	-1896 ± 205 ^a
T5	69 ± 3 ^c	64 ± 4 ^a	45 ± 2 ^b	41 ± 3 ^a	-1851 ± 178 ^b	-1873 ± 252 ^a
	Rs at 10 °C (µgC g ⁻¹ soil h ⁻¹)		Rs at 20 °C (µgC g ⁻¹ soil h ⁻¹)		Rs at 30 °C (µgC g ⁻¹ soil h ⁻¹)	
	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
T1	0.08 ± 0.02 ^a	0.10 ± 0.01 ^a	0.43 ± 0.04 ^a	0.35 ± 0.04 ^a	1.24 ± 0.11 ^a	0.91 ± 0.10 ^a
T3	0.19 ± 0.02^b	0.10 ± 0.02^a	0.60 ± 0.04^b	0.39 ± 0.04^a	1.40 ± 0.11 ^a	1.08 ± 0.11 ^a
T5	0.11 ± 0.01 ^a	0.12 ± 0.02 ^a	0.41 ± 0.04 ^a	0.44 ± 0.05 ^a	1.12 ± 0.10 ^a	1.13 ± 0.14 ^a
	Q_{10} at 10 °C		Q_{10} at 20 °C		Q_{10} at 30 °C	
	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
T1	5.58 ± 0.28^a	3.90 ± 0.24^a	2.94 ± 0.12 ^a	2.66 ± 0.10 ^a	1.69 ± 0.07^a	1.92 ± 0.06^a
T3	3.45 ± 0.28 ^b	4.17 ± 0.28 ^a	2.41 ± 0.12^b	2.93 ± 0.12^a	1.77 ± 0.07^a	2.15 ± 0.07^b
T5	4.00 ± 0.24 ^b	3.72 ± 0.35 ^a	2.82 ± 0.10 ^a	2.65 ± 0.14 ^a	2.08 ± 0.06 ^b	1.97 ± 0.08 ^{ab}
	dRs/dT at 10 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)		dRs/dT at 20 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)		dRs/dT at 30 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	
	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated
T1	0.016 ± 0.002 ^a	0.014 ± 0.002 ^a	0.058 ± 0.005^a	0.039 ± 0.004^a	0.095 ± 0.009 ^a	0.072 ± 0.008 ^a
T3	0.025 ± 0.002^b	0.015 ± 0.002^a	0.060 ± 0.005 ^a	0.046 ± 0.005 ^a	0.099 ± 0.009 ^a	0.092 ± 0.009 ^a
T5	0.016 ± 0.002 ^a	0.018 ± 0.002 ^a	0.047 ± 0.004 ^a	0.049 ± 0.006 ^a	0.098 ± 0.008 ^a	0.089 ± 0.011 ^a

T1 = 1 day, T3 = 125 days and T5 = 349 days after labelling ended.

For MMRT parameters T_{opt} or T_{inf} and ΔC_p^\ddagger , significant differences were observed between dryland and irrigated treatments for all MMRT parameters at Time 1, for T_{inf} at Time 3, but by Time 5, none of these were significantly different (Table 4.5). Comparison of respiration rates from different treatments showed no significant differences at Time 1. At Time 1, the effects of treatment were significant at 10 °C and 30 °C for Q_{10} and at 20 °C for dR_s/dT . At Time 3, there was significance at 20 and 30 °C for Q_{10} . At Time 5, there were no circumstances where treatment was a significant factor in respiration rate.

Very few significant differences in MMRT parameters and Q_{10} at Time 5 suggested that by 349 days, respiration rates from different substrate pools, labelled initially under dryland and irrigated treatments, had converged under the subsequent chase period with a common water regime. This diminishing difference was also highlighted when comparing values between sampling times (Table 4.5). Predicted values for all calculated parameters and values from the irrigated soil were not statistically different across sampling times, whilst for predicted dryland values, Time 1 was often significantly different than Time 5.

Overall, results suggest that temperature response (MMRT parameters) of respiration rates were influenced by treatment type and sampling time. The most apparent difference was that MMRT parameters were different in the first sampling time, but mainly for the dryland treatment. The source of carbon (NPC and SOC) may be a significant factor in the rate of respiration and its temperature sensitivity (Q_{10} and dR_s/dT).

Root material vs shoot material

In addition to measuring respiration rate of labelled soils, the respiration rates of a control soil (under irrigated or dryland conditions but with no ^{13}C labelling) with added enriched herbage litter, or root material from treated mesocosms, was measured and analysed (Table 4.6).

Table 4.6: Calculated MMRT parameters, Q_{10} and dRs/dT (mean \pm std) derived from the respiration of a Limore stony loam soil, mixed with ^{13}C enriched root or shoot litter, incubated for 5 hours. No statistical comparisons were made*.

	Treatment	C source	T_{opt} (°C)	T_{inf} (°C)	ΔC_p^{\ddagger} (J mol ⁻¹ K ⁻¹)	Rs at 10 °C ($\mu gC\ g^{-1}soil\ h^{-1}$)	Rs at 20 °C ($\mu gC\ g^{-1}soil\ h^{-1}$)	Rs at 30 °C ($\mu gC\ g^{-1}soil\ h^{-1}$)	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dRs/dT at 10 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 20 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)	dRs/dT at 30 °C ($\mu gC\ g^{-1}soil\ h^{-1}\ ^{\circ}C^{-1}$)
Root added	Dryland	Litter	42 \pm 2	22 \pm 1	-2144 \pm 255	0.04 \pm 0.01	0.09 \pm 0.01	0.15 \pm 0.01	2.27 \pm 0.13	1.61 \pm 0.05	1.19 \pm 0.04	0.004 \pm 0.001	0.006 \pm 0.001	0.005 \pm 0.001
		SOC	n/a**	n/a**	n/a**	0.32 \pm 0.02	0.90 \pm 0.13	2.20 \pm 0.33	2.76 \pm 0.24	2.45 \pm 0.07	2.20 \pm 0.16	0.035 \pm 0.006	0.086 \pm 0.017	0.184 \pm 0.022
	Irrigated	Litter	58 \pm 32	31 \pm 15	-2002 \pm 1850	0.02 \pm 0.01	0.05 \pm 0.01	0.09 \pm 0.01	2.29 \pm 0.51	1.64 \pm 0.05	1.26 \pm 0.32	0.002 \pm 0.001	0.003 \pm 0.001	0.003 \pm 0.002
		SOC	108 \pm 24	70 \pm 15	-904 \pm 315	0.33 \pm 0.05	1.04 \pm 0.03	2.69 \pm 0.08	3.16 \pm 0.35	2.59 \pm 0.16	2.17 \pm 0.03	0.041 \pm 0.001	0.108 \pm 0.006	0.231 \pm 0.017
Shoot added	Dryland	Litter	39 \pm 1	20 \pm 1	-2115 \pm 142	0.11 \pm 0.01	0.24 \pm 0.01	0.36 \pm 0.01	2.15 \pm 0.08	1.51 \pm 0.03	1.11 \pm 0.01	0.011 \pm 0.001	0.014 \pm 0.001	0.009 \pm 0.001
		SOC	65 \pm 11	33 \pm 6	-982 \pm 204	0.81 \pm 0.04	1.60 \pm 0.06	2.65 \pm 0.05	1.98 \pm 0.05	1.66 \pm 0.04	1.42 \pm 0.06	0.063 \pm 0.004	0.095 \pm 0.003	0.113 \pm 0.008
	Irrigated	Litter	38 \pm 2	18 \pm 1	-2115 \pm 334	0.21 \pm 0.01	0.42 \pm 0.04	0.61 \pm 0.06	2.01 \pm 0.11	1.45 \pm 0.01	1.08 \pm 0.04	0.018 \pm 0.002	0.022 \pm 0.003	0.013 \pm 0.001
		SOC	76 \pm 21	41 \pm 12	-837 \pm 280	0.60 \pm 0.03	1.20 \pm 0.05	2.05 \pm 0.02	2.01 \pm 0.01	1.71 \pm 0.06	1.49 \pm 0.10	0.047 \pm 0.003	0.073 \pm 0.001	0.095 \pm 0.010

*MMRT fit produced was *low replication (n=2) imposed by having little source material

**MMRT fit produced was Arrhenius in nature, and thus these variables could not be calculated

Statistical comparisons of root material respiration rate (Root-Rs) and herbage material respiration rate (Shoot-Rs) were constrained by limited root and shoot material remaining from the Carmona (2020) experiments that would otherwise allow greater replication. There was also some difficulty with adequately distributing minimal amounts of material throughout the soil sample, and some replicates had very large outliers, so the comparisons made here are observational only but align well with our previous work (Robinson et al., 2020). Overall, Root-Rs produced slightly less CO₂ than Shoot-Rs, but MMRT parameters appeared very similar between litter source (root and shoot) for both irrigated and dryland treatments (T_{inf} ranged from 18 to 30 °C). SOC-Rs, MMRT parameters varied but were always substantially higher than the Root/Shoot-Rs. Q_{10} declined with temperature increases as expected and was greater for samples with added root inputs, as was dRs/dT (Figure 4.6).

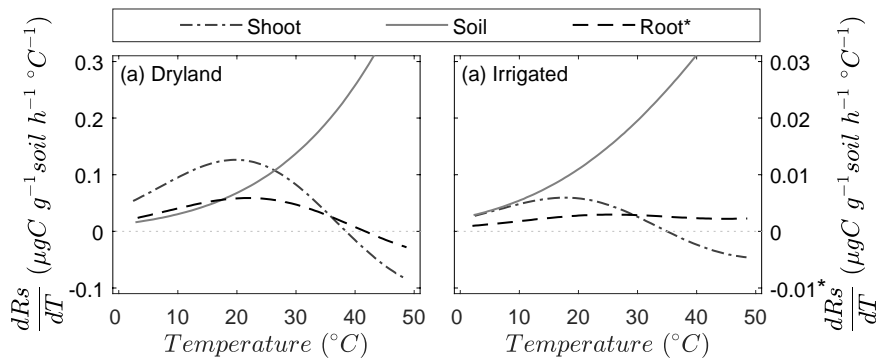


Figure 4.6: *Note that root data for both treatments are measured on the 2nd y-axis. First derivative (dRs/dT) calculated from MMRT model fits of respiration rates from a five-hour incubation of a Limore soil (60% MWHC), mixed with a ¹³C enriched root, or shoot litter. Litters were labelled under (a) dryland or (b) irrigated treatments; unlabelled soils were also treated the same. Total respiration has been partitioned into respiration sourced from litter carbon or bulk soil organic carbon. Different lines represent average temperature sensitivity ($n = 3$) for respiration from root litter, shoot litter and bulk soil organic carbon.

4.5 Discussion

Temperature response of root and shoot respiration.

Additions of labelled shoot material to un-labelled soil produced similar temperature responses to Robinson et al. (2020), which also used a similar ryegrass/clover labelled litter mixed with a Horotiu silt loam. Here, shoot and root T_{inf} values ranged from 18 to 30 °C, while in Robinson et al. (2020), litter additions resulted in a T_{inf} with a similar range of 19 to 24 °C. In all cases, respiration from litter additions had greater curvature (more negative ΔC_p^\ddagger , lower T_{inf}) than soil respiration, which sometimes showed no T_{opt} or T_{inf} and thus had an almost entirely Arrhenius response, as was also observed in Robinson et al. (2020). Similar MMRT parameters suggested that in this situation, roots and shoots have similar decomposability with respect to temperature, but respiration rates from shoot material were greater than from root material.

The temperature response of added litter supported Robinson et al. (2020) and Schipper et al. (2019). They previously hypothesised that the temperature sensitivity of biological decomposition of soil carbon, when substrate is less limiting, is dependent on enzyme activity which generally displays a distinct temperature optimum that is well described by an MMRT response curve (Schipper et al., 2019). When substrate is more limiting, respiration rates are more dependent on desorption and diffusion of substrate (controlled by physical chemistry) to the microorganisms resulting in a more Arrhenius response curve. Systems that contain both limitations (enzymatic and physical chemistry constraints) will result in a mixed temperature dependence that reflects the balance between these two processes, allowing comparison of what process may be contributing to a temperature response (Alster et al., 2020; Schipper et al., 2019).

Systems with more of a reliance on diffusion and sorption/desorption processes will have higher T_{opt} and T_{inf} , while those with greater biological availability (not restricted by chemical or physical protection) of substrate supply will have lower T_{opt} and T_{inf} (Alster et al., 2020; Robinson et al., 2020; Schipper et al., 2019).

The similar MMRT parameters for shoot and roots suggested that the portion of carbon available for decomposition from shoots and roots had the same temperature response. However, there was potentially a limitation on the absolute rate of root-derived respiration and relative/absolute temperature sensitivities. Fulton-Smith and Cotrufo (2019) compared decomposition of *Sorghum bicolor* stalk and root residuals to determine whether the increased chemical recalcitrance of the root material would lead to changes in carbon contribution and allocation within the bulk soil. They found that stalk residues, which they considered less recalcitrant, were decomposed significantly faster than root residuals. Differences in recalcitrance or litter quality may explain the differences we observed in respiration rate between root and shoot material. However, it appears in our case that this potential difference in recalcitrance did not alter the shape of the temperature response of decomposition of root and shoots. Rasse et al. (2005), when comparing incubations studies of root and shoot tissues, generally found roots were retained in soil to a greater degree than shoot inputs. They suggested that higher chemical recalcitrance of root material only contributed to 1/4 of root retention in the soil, and other root interactions, such as physical and chemical protection, played a larger role in stabilisation (Rasse et al., 2005). Kong and Six (2010) similarly found that root carbon, which was more chemically recalcitrant, accumulated up to three times more readily than residue carbon in soil and suggested both quantity and type of carbon source was important in stabilisation. Overall these results showed that decomposition root and shoot litters likely had the same response to temperature but

had different absolute rates of respiration, which appears to support the suggestion that physical or chemical protection, not recalcitrance, is the determining factor in the temperature response of soil decomposition (Cotrufo et al., 2013; Dungait et al., 2012; von Lützow & Kögel-Knabner, 2009).

Temperature response of new photosynthate carbon and soil organic carbon

In contrast to the stark difference between T_{inf} of root litter-derived respiration and soil-derived respiration, within sampling times or treatments, there were very few significant differences between the MMRT parameters for NPC-Rs and SOC-Rs. In other words, the temperature response of carbon added through growing roots was generally indistinguishable from soil organic matter for both irrigated and dryland treatments.

Respiration rates of NPC-Rs accounted for a little less than half of the total respiration, despite this newly added carbon only accounting for ~5% of total carbon in the bulk soil on average (Carmona et al. 2020). Similar respiration rates of NPC-Rs and SOC-Rs suggested that two carbon sources had roughly equal amounts of carbon available for degradation. When considering total carbon, the higher respiration rate per proportion of total carbon indicated that more of the new photosynthate carbon was available for decomposition relative to total SOC. We could assume, as measured respiration rates were similar, a large proportion of SOC is very biologically unavailable, and only a small proportion, similar to the size of the new photosynthate proportion, contributed to the observed temperature response. This suggestion is well supported in the literature where large proportions of carbon (e.g. 53% as mineral associated organic carbon in Benbi et al. (2014); 48-65% carbon associated with the

fine-silt/clay fraction in Larionova et al. (2015); 80 to 93% carbon associated with the fine fraction in McNally et al. (2018) are found in soil fractions that are often associated with protected/stabilised or slow-cycling carbon (Six et al., 2002; Wiesmeier et al., 2019), and may not be contributing substantially to short-term soil respiration.

Similar values for T_{inf} and T_{opt} for NPC-Rs and SOC-Rs supported the hypothesis that there was a similar balance between sorption/desorption and enzyme activity controlling the decomposition of each carbon pool. This similar balance suggests either of two possibilities, 1) that NPC has entered the soil at a similar stage of degradability to SOC or 2) within three months during the labelling ^{13}C -CO₂ phase, new carbon has been quickly stabilised in soil and reached a similar state of degradability. These results support a study by Sokol et al. (2019a) where living root inputs to soil were found to be up to 13 times more efficient in forming SOC than root/shoot litter inputs. Although they could not fully distinguish whether the differences were due to the chemical composition of living root inputs, or the differences in the pathway of input into soil, it seems in general that living root inputs were more likely to be stabilised in SOC compared to mass litter inputs of either roots or shoots (Sokol et al., 2019a).

Comparisons of temperature response under Irrigated and Dryland treatments.

For Time 1, there was a significant difference between total dryland and irrigated soil MMRT parameters, and temperature sensitivity measures and irrigated respiration rates were lower than dryland, significantly so at Time 3. These results were comparable to results described in Schipper et al. (2019), where soil respiration rate was significantly less under pivot irrigation with a T_{opt} and T_{inf} of 70.2 and 45.5 °C,

respectively, compared to respiration rate for adjacent, non-irrigated soil with a T_{opt} and T_{inf} of 53.7 and 32.5 °C respectively.

Schipper et al. (2019) argued that the lower respiration rate under irrigation was indicative of less carbon available for microbial decomposition rather than the decline in total carbon measured by Mudge et al. (2017). This decline in carbon availability was also used to explain differences in temperature sensitivity (high Q_{10} /low dR_s/dT) and higher T_{opt} and T_{inf} as respiration in these systems was more dependent on diffusion/desorption than non-irrigated soils. Carmona et al. (2020b) showed that irrigation and dryland treatments did not affect total carbon, which, when combined with the reported higher temperature sensitivity with irrigation treatment, supported the hypothesis that the availability of carbon, rather than total carbon stock was important for temperature response of decomposition. Further still, Carmona et al. (2020b) reported that although net storage of new carbon in the non-rhizosphere soil was similar between treatments when fractionated, there was more storage of new carbon in the $< 5 \mu m$ fraction under irrigation (expressed as a proportion of total new carbon storage). Carbon associated with $< 5 \mu m$ MAOM fraction (mineral associated organic matter) is typically considered to be stable or slow cycling carbon (Six et al., 2002; Wiesmeier et al., 2019). A greater proportion of new carbon inputs in this fraction could indicate a change in carbon stabilisation by biological processing, which has been reported to increase under irrigation (Scott et al., 2012) through means such as increased earthworm activity (Fraser et al., 2012). However, ultimately the comparative mechanisms of carbon storage within irrigated and non-irrigated soils remain poorly understood (Whitehead et al., 2018).

An increase in the stabilisation of carbon inputs under irrigation (reduced respiration) would explain the apparent treatment effect and reduction in available carbon (Time 3) as reported here, although this does not describe what effects irrigation may have on total carbon. As Mudge et al. (2017) reported a decrease in total carbon under irrigation from the same locations, it may be that over short periods, irrigation does lead to apparent increases in carbon stabilisation; however, with time sustained high rates of biological activity and turnover results in eventual losses of total carbon.

Changes in temperature response over time, under constant conditions.

As the post-treatment chase period commenced and soils were subjected to the same moisture contents, the initial significant differences in temperature responses between treatments subsided. This result suggested that soil carbon formed under dryland and irrigated conditions were different initially, but when the same conditions were applied, the temperature response of respiration becomes the same. As all measured irrigated MMRT parameters and Q_{10} values did not change with time (Time 1 – Time 5), it can be assumed that under initially dryland soils, carbon cycling responded to subsequent increases in moisture content over the following year. By Time 5, processes that controlled turnover and potential stabilisation of substrate under the original dryland conditions changed in response to increased water availability whilst the irrigated treatment remained at equilibrium.

These results align with the findings by Carmona et al. (2020a), who observed the allocation and persistence of new carbon inputs using the same set of enriched soils. Carmona (2020) found no significant difference in the total mass of ^{13}C , recovered in the non-rhizosphere soil (0-15cm) between treatments, and no change over the

sampling period. However, summer irrigation appeared to alter carbon allocation and storage in the soil when observing ^{13}C in different soil fractions (Carmona et al., 2020a). Total ^{13}C mass in the three largest fractions, coarse POM ($> 250 \mu\text{m}$), fine POM ($53\text{--}250 \mu\text{m}$) and clay ($< 5 \mu\text{m}$) fractions, did not change with time for initially irrigated soils, yet varied for dryland soils (Carmona et al., 2020a). At Time 1, previously dryland soils had significantly less ^{13}C in the clay and fine POM fractions. By Time 3, ^{13}C in the coarse POM and clay fractions increased significantly above irrigated values in the case of coarse POM. However, by Time 5, the ^{13}C in all three fractions was statistically the same (Carmona et al., 2020a). It was suggested that the higher ^{13}C in the clay and fine POM fractions under initial irrigation, paired with the considerable movement of ^{13}C in previously dryland soils under subsequent higher moisture contents at Time 3 and Time 5, could be indicative of a more efficient pathway of photosynthate C into the smaller fractions due to increased root turnover.

Temperature sensitivity

Like temperature response, measures of temperature sensitivity showed a slight difference between dryland and irrigated treatments which diminished through between Time 1 and Time 5 when soils were maintained under similar conditions. A higher temperature sensitivity (Q_{10}) under irrigated conditions was previously attributed to less substrate available in irrigated soils (Schipper et al., 2019), although this difference was not as pronounced in this study. Carbon source (NPC-Rs or SOC-Rs) appeared to significantly influence on temperature sensitivity, with NPC-Rs appearing to be less temperature sensitive than SOC-Rs in most cases (higher Q_{10} , lower dRs/dT). This difference in temperature sensitivity may suggest that NPC was more recalcitrant than SOC which would contradict the concept that older carbon was

likely more chemically recalcitrant (Conant et al., 2011). Therefore, these results may further support theories, such as the MEMS framework, that old carbon is not inherently more recalcitrant but rather more stabilised (Cotrufo et al., 2013; Dungait et al., 2012).

4.6 Conclusions

Overall, there were very few significant differences between temperature response of respiration from new photosynthate carbon or SOC within irrigated or dryland treatments, yet respiration derived from added root and shoot litter was considerably different to SOC-Rs. Similarities between the temperature response of NPC and SOC were attributed to living root inputs being quickly stabilised in soil similar to older inputs of carbon SOC or entering the system at a similar state of degradability compared to that of litter. There was some evidence of recalcitrance altering temperature sensitivity; however, this was inconsistent throughout sampling times and moisture treatments. Comparison of dryland and irrigated respiration response to temperature showed that irrigated respiration had a lower T_{opt} and T_{inf} comparatively. These effects were comparable to Schipper et al. (2019) and were attributed to a decline in available carbon under irrigation due to increased stabilisation by root-turnover, leading to a more Arrhenius-like response. Dryland effects diminished between 125 and 349 days of constant autumnal conditions suggesting microbial adjustment to increased water availability occurs relatively quickly. In general, root carbon inputs appeared much more stable than additions of ground litter, and so efforts to increase carbon stabilisation in soil should focus on inputs through growing roots rather than additions of plant fragments such as above-ground litter.

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Conflict of Interest

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Chapter 5. Short-term effects of irrigated and dryland treatments on the temperature response of respiration from soil organic matter and new photosynthetic carbon.

Jasmine M. Robinson^{a*}, Carmen R. Carmona^{bc}, Samuel R. McNally^c, Michael H. Beare^c, Louis A. Schipper^a.

^aThe University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand

^bDepartment of Soil and Physical Sciences, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand.

^cPlant & Food Research, Private Bag 4704, Christchurch Mail Centre, Christchurch 8140

The contributions of authors were (see co-authorship form at back of thesis):

Jasmine Robinson, Louis Schipper and Mike Beare decided on experimental design.

Carmen Carmona and Sam McNally contributed enriched soils and litter for analysis.

Jasmine Robinson was responsible for running experiments, statistical analyses and writing the manuscript. Louis Schipper was the primary reviewer for this manuscript.

This chapter has not been published.

5.1 Abstract

To fully understand the effects of irrigation on the temperature response of soil respiration, both the analysis of the irrigation effect on plant inputs of carbon to soil and the effects of irrigation on existing carbon stores, is essential. We use a recently developed method to measure the effects of seasonal irrigation application on the temperature response of soil respiration derived from soil organic carbon (SOC) and newly deposited photosynthate carbon (NPC), labelled with ^{13}C under similar moisture conditions. Within treatments, respiration from NPC and SOC had the same temperature response (T_{inf} of 38 and 39 °C, respectively). Additionally, the temperature responses of soil respiration between subsequent irrigated or dryland treatments were not significantly different (Time 3; averaged T_{inf} of 40 and 39 °C, respectively).

The similarities in the temperature response between irrigation and dryland treatments indicate that short-term seasonal irrigation (three months) did not significantly impact carbon availability in the soil. The similarity of NPC and SOC suggested new inputs of carbon enter the system at similar availability as older carbon or is quickly stabilised. Overall, we suggest differences in the temperature response of soil respiration seen in field studies under irrigated or dryland management are likely result from increased initial stabilisation of live plant inputs rather than later carbon cycling in the soil. Future research centred around carbon stabilisation should prioritise studies on the management of live root inputs.

5.2 Introduction

Carbon exists in many pools in soil, and at their most basic, are distributed between available and more protected/stored pools (Conant et al., 2011; Lal, 2018). Due to the complexity of soil carbon dynamics, it is reasonable to suggest that the effects of management practices and environmental drivers, including changes to moisture and temperature, may differ for these different pools of carbon (Conant et al., 2011; Davidson & Janssens, 2006). Irrigation of pasture is an important management practice that increases plant growth and food production; however, there are concerns about whether irrigation leads to increases in CO₂ production and losses of carbon stocks (Lal, 2018; Whitehead et al., 2018). Increasing the understanding of soil carbon storage and identifying management practices that can maintain or increase soil carbon without limiting food production will be valuable for future food and climate security (Whitehead et al., 2018).

The consequences of irrigation on soil carbon stocks and dynamics are poorly understood (Trost et al., 2013). However, in a comprehensive study measuring changes in carbon stocks under irrigation, Mudge et al. (2017) sampled 34 paired irrigated and non-irrigated sites across New Zealand that ranged from 3 – 90 years under seasonal irrigation. They showed that, on average, there was 6.99 t C ha⁻¹ less carbon in the top 0.3 m soil under irrigation compared to adjacent dryland sites. They suggested that increased water content could encourage leaching of existing soil carbon and increase microbial decomposition leading to declines in soil carbon with time (Mudge et al., 2017).

While full temporal changes were not fully explored, Mudge et al. (2017) suggested that declines in soil carbon occurred rapidly in the first few years after the application of seasonal irrigation. They argued that one possible reason was increased water availability during warm summers accelerating microbial activity and decomposition of soil organic matter (Mudge et al., 2017). Similarly, in a temporal analysis of soil carbon stocks over 50 years of seasonal irrigation at Winchmore Irrigation Research Station, Schipper et al. (2013) showed that irrigation resulted in a lower soil carbon content (0–10 cm) in the first few years. These studies suggest rapid loss of carbon under irrigation commences on dryland pastures; however, the underlying mechanisms which drive these changes were not fully understood.

Others have initiated studies of microbial cycling of different pools of soil carbon under irrigation. In a study that aimed to observe the allocation, and subsequent cycling, of carbon under seasonal irrigation, Carmona (2020) used ^{13}C -CO₂ to enrich new photosynthate carbon inputs to soil under different regimes. When plants were initially grown under irrigated and dryland conditions, Carmona (2020) found that seasonal irrigation did not change the total amount of new carbon recovered from soils than dryland treatments; however, irrigation did alter the allocation of carbon into different soil fractions. More new carbon was found in the fine particulate organic matter (POM; 53–250 μm) and clay (< 5 μm) fractions in irrigated soils compared to dryland treatments. Additionally, in a second experiment by Carmona (2020), soils were labelled with ^{13}C under the same spring moisture content, after which three months of seasonal irrigation or dryland conditions were applied. They showed that, although total new carbon was not changed, after three months, dryland soils had more new carbon present in the non-rhizosphere soil compared to irrigated soil, a large proportion of which was in the clay fraction. Under irrigation, more new carbon was

retrieved in the lower profile (15–25 cm). The enhanced flow of carbon under irrigation into the stable fractions in the first experiment and this apparent movement of carbon into the lower profiles in the second experiment were both attributed to increased root turnover, aggregation, and potential short-term stabilisation of carbon under irrigated pasture due to increased biological activity under irrigated treatments (Carmona et al., 2020).

While studying carbon inputs is important, of equal importance is understanding microbial decomposition/respiration of different carbon pools in soil and how decomposition may respond to changes in variables, such as temperature (Conant et al., 2011; Davidson & Janssens, 2006). In a previous study, Robinson et al. (2020) examined the temperature response of microbial respiration from both soil organic matter and an added ^{13}C labelled litter. Respiration rates were fitted with macromolecular rate theory (MMRT), which allowed calculation of the parameters T_{inf} (the temperature at which the change in rate of respiration is maximal) and T_{opt} (the temperature where respiration rate is maximal; Alster et al., 2020; Schipper et al., 2014). These parameters examine more biochemically relevant parameters and may provide further insights into microbial mediated processes occurring during decomposition (Alster et al., 2020). Robinson et al. (2020) found that these metrics of temperature response (T_{opt} and T_{inf}) of litter were lower than that of soil organic carbon, which was attributed to the relative availability of litter carbon compared to soil organic carbon (Robinson et al., 2020). This work was expanded in Chapter 4, where the temperature response was measured for both soil organic matter and new enriched inputs of photosynthate carbon, labelled under irrigated and dryland conditions (from Carmona, 2020). Here, new photosynthate carbon had a similar temperature response (T_{opt} and T_{inf}) to soil organic carbon, which was indicative of new inputs being rapidly

incorporated into soil and achieving similar availability or having a similar carbon quality upon entry to the soil (Robinson et al., 2020).

Our main objective was to determine whether the establishment of irrigation on a previously dryland pasture soil alters the cycling of carbon and the temperature of soil respiration from soil organic matter (SOC) and newly deposited photosynthate sources (NPC; enriched carbon sourced from root exudates and root/shoot derived litter). If carbon cycling was accelerated following irrigation, this might explain the observed lower soil carbon under irrigation. ^{13}C enriched soil and litter samples were acquired from the above study by Carmona (2020) and incubated with the resulting CO_2 analysed for its isotopic signature using the method described by Robinson et al. (2020). The isotopic signature allowed the determination of separate temperature responses from both the new photosynthate carbon and old carbon sources from one respiration measurement. We hypothesised that there would be no difference in the temperature response of NPC and SOC as carbon inputs will be of the same availability. In terms of differences between dryland and irrigated treatments, due to increased stabilisation and cycling under irrigation, we expected that respiration rates under irrigation should be less than that of dryland. If the hypothesis, suggested by Schipper et al. (2019), that the temperature response of the biological decomposition of carbon is assumed to apply here, a reduction in available carbon for decomposition will be seen in the temperature response. We, therefore, expected to see a different temperature sensitivity (higher Q_{10}) under irrigation compared to dryland soils.

5.3 Methodology

Soil sampling and labelling

For this experiment, soils were supplied by Carmona (2020), where full experimental details can be found. In summary, 48 microcosms ($\sim 15 \times 25$ cm round), containing a reconstructed Lismore stony silt loam soil profile (Udic Ustochrept, 0-15 cm, collected from an established dryland Lucerne pasture at Ashley Dene Research and Development farm, Lincoln, Canterbury, NZ), were established with pasture over five months under constant winter-spring conditions with periodic fertilisation (Carmona, 2020).

Once pasture had established, 48 microcosms were placed, in a randomised block design ($n = 4$), into a large chamber (sealed transparent acrylic, 2 m length \times 1.2 m width \times 1 m height). Over a three-month labelling period, daily additions of evolved $^{13}\text{CO}_2$ (equivalent to 0.5 g $\text{Na}_2^{13}\text{CO}_3$) delivered from a gasbag using a precision air pump into the chamber. Total CO_2 , $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ concentration were continuously monitored via a data logger (Campbell Scientific CR1000, USA) and a cavity ring down spectrometer (Picarro G2210-i, Santa Clara, CA). Background CO_2 concentration within the chamber was maintained at ~ 400 ppm (Carmona, 2020).

During the labelling period, soil moisture was maintained at typical *in situ* spring-conditions ($\sim 18 - 32\%$ volumetric water content; VWC), after which microcosms were assigned one of two treatments, dryland conditions ($\sim 7 - 20\%$ VWC) or irrigated ($\sim 24 - 33\%$ VWC) conditions (Carmona, 2020). These two treatments were applied for three months during the summer period; following this, all mesocosms were maintained at

similar seasonal moisture contents (between 24 – 40% VWC). At five time periods after labelling (Time 1 = 1 day, Time 2 (Application of Irrigated and Dryland treatments) = 15 days, Time 3 (Application of autumnal conditions) = 140 days, Time 4 = 225 days, Time 5 = 343 days) during this experiment, root and shoot samples were taken, and destructive sampling was completed (0 – 15 cm and 15 - 25 cm depths) for analysis of ^{13}C ‰, total carbon and total nitrogen of rhizosphere for both non-rhizosphere and bulk soil.

For the current study, four replicate microcosms, from each treatment, at three sampling times were selected that had sufficient residual soil (Time 1, Time 3 and Time 5) to determine temperature response of respiration as an indication of changes in carbon cycling (Figure 5.1).

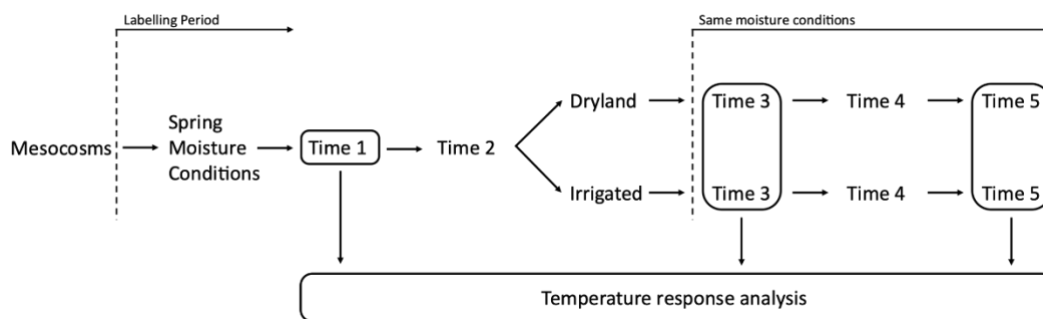


Figure 5.1: Timeline of the three-month ^{13}C labelling process and subsequent chase period by Carmona (2020). Sample times 1 (1 day after labelling), 3 (140 days after labelling), and 5 (343 days after labelling) were sub-sampled for temperature response analysis.

Soil was also collected from four control (non-labelled) microcosms from Time 1 and two from both dryland and irrigated treatments from Time 5 to use as a reference of normal CO_2 production (Appendix 3). In total, there were 28 soil samples, four labelled

replicates from Time 1 before irrigation/dryland treatment was imposed, four labelled replicates from each treatment, at Time 3 and Time 5 sampling times (20 total), and eight unlabelled replicates. Carmona (2020) provided all values for soil ^{13}C ‰, total carbon and root mass values (Table 5.1).

Table 5.1: Root ^{13}C ‰ values and bulk soil total carbon for sampled mesocosms, values from Carmona (2020).

		Mesocosm I.D.	Root enrichment (^{13}C ‰)	Total carbon (%)
Time 1	Spring MC	9	149.46	3.04
		24	197.44	3/06
		29	207.99	3.08
		44	227.67	3.07
Time 3	Dryland	5	107.77	3.17
		23	118.65	3.16
		36	124.16	3.08
		47	119.00	3.21
	Irrigated	14	58.48	3.18
		13	21.44	3.11
		33	19.41	3.14
		37	20.28	3.13
Time 5	Dryland	3	20.23	3.02
		21	18.61	3.04
		26	29.44	3.02
		41	16.22	2.92
	Irrigated	6	-10.64	3.00
		15	-7.45	2.95
		31	-11.47	2.94
		39	-14.20	3.01

Temperature sensitivity analysis.

Seven days before incubation, soils were wet up to 60% maximum water holding capacity (MWHC) as calculated in Harding and Ross (1964), equivalent to ~28% VWC. Soils were subsequently weighed (4 g) into 30 Hungate tubes (38 mL) and incubated for five hours between ~2 and 50 °C. After incubation, to halt further respiration, the Hungate tubes were immediately placed into ice and stored at -20 °C overnight for subsequent ^{13}C -CO₂ analysis.

^{13}C -CO₂ analysis used a modified Los Gatos OA-ICOS CO₂ to analyse headspace gas samples, as described in Robinson et al. (2020), with a carrier gas adjustment. A mix of five internal standards (range of bicarbonate solutions from ~ 30 ‰ to 250 ‰, standardised using the international reference carbonate standards) and international standards (NSB-1: 1.95 ‰, sigma: -14.18 ‰, BDH: -24.95 ‰, as reported in Beinlich et al. (2017) were analysed alongside samples to allow reporting of values to $\delta^{13}\text{C}_{\text{VPDB}}$. Replication of standards reported an error of ~0.05‰.

Data analysis

A 2-pool mixing model (Equation 5.1) was used to derive both the new photosynthate carbon (^{13}C labelled) and soil organic carbon derived CO₂ from the total CO₂ produced during the incubation period.

$$f = \frac{(C_S - C_C)}{(C_R - C_C)} \quad \text{Equation 5.1}$$

Where C_s , C_C , and C_R represent the soil, total respired CO_2 and the root (representative of the NPC) $\delta^{13}C_{VPDB}$ values, respectively.

Application of Equation 5.1 resulted in three individual temperature-respiration (R_s) curves:

- (a) combined soil + new photosynthate (Tot- R_s),
- (b) new photosynthate carbon-derived (NPC- R_s),
- (c) soil organic carbon-derived (SOC- R_s).

MMRT (Equation 5.2; Hobbs et al., 2013; Schipper et al., 2019) was fitted to each of these curves individually. From these fits, the temperature optimum (T_{opt}) and inflection point (T_{inf}) were then calculated using Equations 5.3 and 5.4. Additionally, Q_{10} was calculated as a measure of temperature sensitivity (Sierra, 2012).

$$\ln(R_s) = \ln \left[\frac{k_B T}{h} \right] - \frac{\Delta H_{T_0}^\ddagger + \Delta C_P^\ddagger (T - T_0)}{RT} + \frac{\Delta S_{T_0}^\ddagger + \Delta C_P^\ddagger (\ln(T) - \ln(T_0))}{R} \quad \text{Equation 5.2}$$

$$T_{opt} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_P^\ddagger T_0}{-\Delta C_P^\ddagger - R} \quad \text{Equation 5.3}$$

$$T_{inf} = \frac{\Delta H_{T_0}^\ddagger - \Delta C_P^\ddagger T_0}{-\Delta C_P^\ddagger \pm \sqrt{-\Delta C_P^\ddagger R}} \quad \text{Equation 5.4}$$

Where ΔC_p^\ddagger (J mol⁻¹ K⁻¹) is the change in heat capacity between the enzyme-substrate complex and the enzyme bound to the transition state (here is representative of the mean ΔC_p^\ddagger of all the enzymes involved in respiration (Schipper et al., 2014), k_B and h are Boltzmann's and Planck's constants. $\Delta H_{T_0}^\ddagger$ is the change in enthalpy (J mol⁻¹), and $\Delta S_{T_0}^\ddagger$ (J mol⁻¹ K⁻¹) is the change in entropy between the enzyme-substrate complex and the enzyme bound to the transition state at a reference temperature (T_0 , which is set to 298 K, 25 °C).

All peak calculations, statistical analyses and data processing were performed using MATLAB R2017b (The MathWorks Inc., Natick, MA, USA). Two-way ANOVA was used to test for differences in parameters between irrigated and dryland results for each sampling time and between sampling times for each treatment. A Tukey's honest significant difference test was used post hoc to compare temperature sensitivity parameters between sampling times. One-way ANOVA was used to compare carbon source parameters within a treatment (irrigation/dryland). For each time period, the same post hoc analysis compared parameters between two specific carbon sources within a treatment.

The use of root isotopic values as the end-point value during partitioning to represent new carbon inputs to soil does have limitations (Figure 5.2). As the chase period extends, shoot, root and soil change to different extents as carbon cycles and new natural abundance carbon enters the system. In this study, root material was assumed to be an appropriate estimation of new carbon inputs to soil at Time 1. At Time 3 and 5, this may not be an accurate representation.

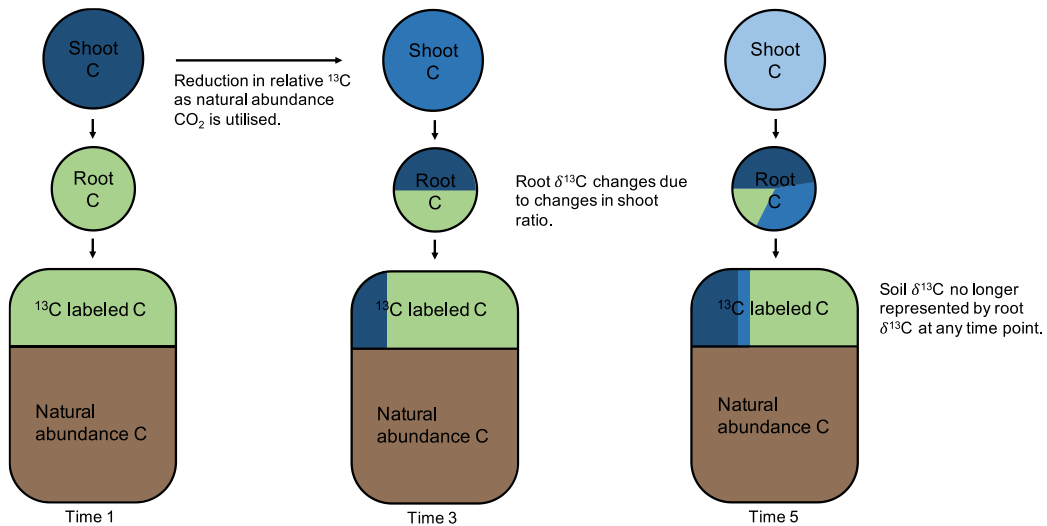


Figure 5.2: Diagram demonstrating how, over time, the isotopic value of shoots, root and new soil carbon changes to different extents. Over time, the isotopic ratio of root carbon will no longer be similar to the isotopic ratio of new carbon inputs to soil.

Using a different end-point should not affect the shape of the respiration curve produced, but the proportion of respiration from the labelled source may be consistently underestimated. This study focuses on comparisons between MMRT parameters rather than measures of temperature sensitivity, as these rely on the curve shape rather than the total amount of CO_2 from each source.

5.4 Results

We compared resulting temperature response curves from soil collected at three sampling times (T1 = 1 day, T3 = 140 days, and T5 = 343 days) after labelling ended (Figures 5.3, 5.4 and 5.5). Soils were initially labelled under constant conditions, then three months of irrigation or dryland treatments were applied, followed by constant moisture conditions for the rest of the year. Measured respiration rates were divided into three CO_2 sources (combined soil + new photosynthate, new photosynthate

carbon-derived (NPC-Rs), and soil organic carbon-derived (SOC-Rs) respiration rate). Comparisons in this section and the discussion will be focused on differences and similarities between the two distinct pools in soil SOM-Rs and NPC-Rs.

Using the isotopic values of root material as a representation of new carbon inputs does come with limitations. For this study, at Time 1, the isotopic value of the root material was assumed an appropriate representation of available new carbon inputs to the soil prior to Time 1. However, as labelled carbon gets redistributed throughout the profile, and root $\delta^{13}\text{C}$ values are diluted by new, natural abundance carbon so that with time, root $\delta^{13}\text{C}$ values may become increasingly less representative of the labelled carbon available in soil at Time 3 and 5. Using different values as end-point at these times would not have affected the shape of the respiration curve produced but rather the proportion of respiration allocated to each source. Thus, we focus comparisons of MMRT parameters and Q_{10} between sampling times, as these rely on the shape rather than the amount of CO_2 from each source.

Respiration rates

As expected, respiration rates, in general, increased with temperature increases and at higher temperatures, showed some curvature to an optimum (Figures 5.3, 5.4 and 5.5).

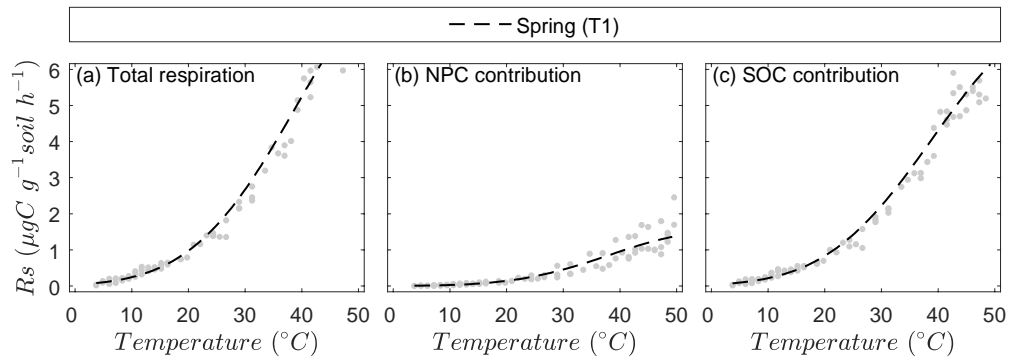


Figure 5.3: Respiration rates (R_s) from a five-hour incubation of a ^{13}C labelled Limore soil (60% MWHC). Soils were sampled one day (Time 1) after labelling under the same conditions. Total respiration (a) was partitioned into new photosynthate carbon (b) and bulk soil organic carbon (c), using a mixing model and fit with MMRT. Symbols (\bullet) indicate three replications of data, while the dashed line represents the average respiration rate.

After the application of either irrigation or dryland treatment over 125 days (total of 140 days after labelling ceased; Time 3), respiration rates under irrigation (Irr- R_s) were typically higher than the equivalent dryland (Dry- R_s) respiration rates (Figure 5.4).

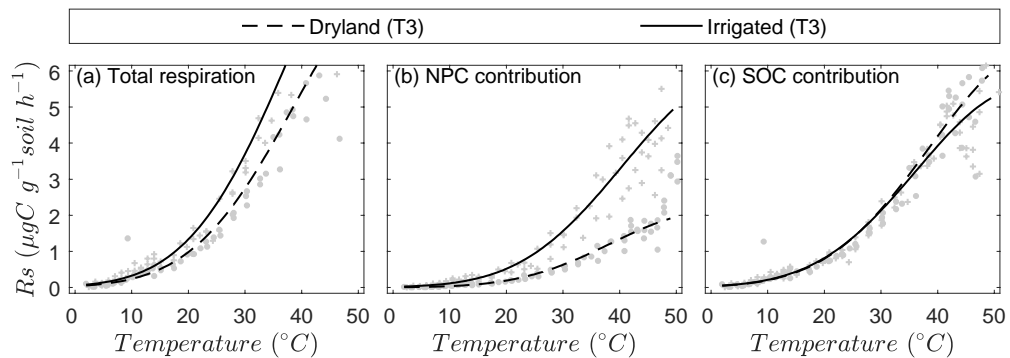


Figure 5.4: Respiration rates (R_s) from a ^{13}C labelled Limore soil (60% MWHC), sampled 140 days (Time 3) after labelling. Soils were incubated for five hours. Total respiration (a) was partitioned into new photosynthate carbon, (b) and bulk soil organic carbon (c) using a mixing model and fit with MMRT. ^{13}C label was applied under the same conditions prior to treatment application. Symbols indicate 4 replications of data for previously dryland (\bullet) and irrigated treatments ($+$). Different lines represent the average response for different treatments.

After irrigation application for 125 days, the same moisture conditions were applied, samples were taken after 203 days of constant conditions, a total of 343 days after labelling ended (Time 5). Variation in respiration rate between replicates was very high compared to previous measurements both in this experiment and previous studies (Figure 5.5).

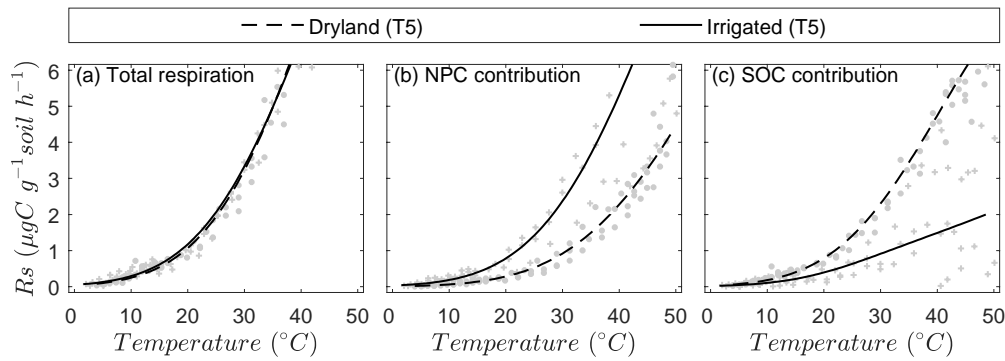


Figure 5.5: Respiration rates (R_s) derived from a five-hour incubation of a ^{13}C labelled Limore soil (60% MWHC), sampled 343 days (Time 5) after labelling. Respiration from new photosynthate carbon (b) and bulk soil organic carbon (c) was partitioned from total respiration (a) from using a mixing model and fit with MMRT. ^{13}C label was applied under the same condition prior to treatment application. Symbols indicate 4 replications of data for dryland (•) and irrigated treatments (+), different lines represent average response for different treatments.

For both treatments, respiration rates from soil organic carbon (SOC- R_s) were generally greater than rates derived from carbon that was deposited by living roots (NPC- R_s ; Figures 5.3, 5.4 and 5.5).

MMRT and temperature sensitivity

Once respiration rates were fitted with MMRT, parameters T_{opt} , T_{inf} and ΔC_p^\ddagger were compared between carbon sources, treatments, and sampling times. For these fitted

parameters, calculated values for NPC-Rs and SOC-Rs were not significantly different within a sampling time and treatment (Table 5.2) except for ΔC_p^\ddagger at Time 1, prior to treatment.

Table 5.2: Calculated MMRT parameters and Q_{10} (mean \pm std, n= 4), derived from a ^{13}C enriched Limore soil, sampled 1 day (Time 1), 140 days (Time 3) and 343 days (Time 5) after labelling.

Treatment	C	T _{opt}	T _{inf}	ΔC _p [‡]	Q ₁₀ at 10 °C	Q ₁₀ at 20 °C	Q ₁₀ at 30 °C	
	source	(°C)	(°C)	(J mol ⁻¹ K ⁻¹)				
Time 1	NPC	56 ± 1	38 ± 1	-2757 ± 79	5.1 ± 0.1	3.2 ± 0.1	2.1 ± 0.1	
	SOC	61 ± 4	39 ± 2	-2061 ± 272	3.9 ± 0.2	2.7 ± 0.1	1.9 ± 0.1	
Time 3	Dryland	NPC	57 ± 3	39 ± 2	-2791 ± 242	5.3 ± 0.2	3.3 ± 0.1	2.1 ± 0.1
		SOC	59 ± 3	39 ± 2	-2162 ± 284	4.0 ± 0.3	2.7 ± 0.1	1.9 ± 0.1
	Irrigated	NPC	65 ± 15	43 ± 10	-2232 ± 736	4.5 ± 0.7	3.0 ± 0.2	2.1 ± 0.2
		SOC	56 ± 3	36 ± 2	-2316 ± 268	3.9 ± 0.4	2.6 ± 0.2	1.8 ± 0.1
Time 5	Dryland	NPC	87 ± 21	60 ± 15	-1526 ± 498	4.4 ± 0.2	3.3 ± 0.1	2.5 ± 0.2
		SOC	61 ± 2	40 ± 1	-2218 ± 131	4.3 ± 0.1	2.9 ± 0.1	2.1 ± 0.1
	Irrigated	NPC	77 ± 19	52 ± 12	-1689 ± 641	4.1 ± 0.8	3.0 ± 0.3	2.2 ± 0.1
		SOC	53 ± 30	34 ± 19	-3810 ± 2606	4.0 ± 0.7	2.2 ± 0.4	1.4 ± 0.7

Respiration (R_s , $\mu\text{g C g}^{-1} \text{ h}^{-1}$) was sampled at the end of a 5 hour incubation period. Bold values indicate the significant differences (< 0.05) between those two carbon sources within a treatment.

From MMRT fits produced, the temperature sensitivity parameter Q_{10} was calculated. When compared at 10, 20 and 30 °C, Q_{10} for NPC-Rs was generally higher than Q_{10} calculated for SOC-Rs, frequently significantly, throughout sampling times (Table 5.2).

Effect of treatment and carbon source on respiration response

Statistical comparisons within individual sampling times were used to investigate the influence different carbon sources and applied treatments have on calculated values (Table 5.3).

Table 5.3: Significance level (p-value) given of main effects and interactions for the comparison of values from both dryland and irrigated treatments, within either Time 3 or Time 5. Significant values are shown in bold.

		T_{opt}	T_{inf}	ΔC_p^{\ddagger}	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C
Time 3	Carbon source	0.517	0.255	0.308	0.006	< 0.001	0.002
	Treatment	0.611	0.708	0.443	0.153	0.074	0.362
	Interaction	0.226	0.240	0.193	0.197	0.334	0.474
Time 5	Carbon source	0.062	0.043	0.112	0.659	0.006	0.014
	Treatment	0.474	0.377	0.298	0.309	0.012	0.052
	Interaction	0.946	0.912	0.391	0.932	0.205	0.358

Carbon source = NPC or SOC, Treatment = irrigation or dryland. Pairwise comparisons were not included in this table as both factors are pairs results of a pairwise comparison would have the same significant differences as above.

For all MMRT parameters at either sampling time, dryland or irrigated treatments or carbon source generally did not have a significant influence on calculated values (Table 5.3). For Q_{10} at Time 3, carbon source was a significant factor for all three temperatures. At Time 5, Q_{10} at 20 and 30 °C, were influenced by carbon source. While treatment only appeared to affect values of Q_{10} at 10 °C. These results indicated that whether respiration came from either NPC or SOC sources significantly influenced the measured Q_{10} but did not affect MMRT parameters for the same sampling time.

Effect of sampling time and carbon source on respiration response

A diminishing difference between irrigated and dryland parameters by Time 5 was highlighted when comparing MMRT parameters and Q_{10} between sampling times and carbon source within dryland or irrigated treatments (Table 5.4).

Table 5.4: Significance level (p-value) given of main effects and interactions for the comparison of values from all sampling times within dryland or irrigated treatment. Significant values are shown in bold. For pairwise comparison, different letters (i.e., a, b, c) note the significant differences (< 0.05) between sampling times within a treatment.

Treatment	T_{opt}	T_{inf}	ΔC_p^{\dagger}	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C
Dryland	Carbon source	0.149	0.061	0.142	< 0.001	< 0.001
	Sampling Time	0.012	0.011	0.006	0.131	0.018
	Interaction	0.016	0.022	0.002	0.142	0.059
	Pairwise (T1, T3, T5)	a, ab, b	a, ab, b	a, ab, b	a, a, a	a, a, b
Irrigated	Carbon source	0.214	0.121	0.371	0.021	< 0.001
	Sampling Time	0.751	0.767	0.765	0.391	0.441
	Interaction	0.306	0.297	0.132	0.242	0.365
	Pairwise (T1, T3, T5)	a, a, a	a, a, a	a, a, a	a, a, a	a, a, a

T1 = Time 1, T3 = Time 3, T5 = Time 5. Carbon source = NPC or SOC, Treatment = irrigation or dryland. Pairwise comparison of carbon source was not included in this table as these parameters are already a pair. Results of a pairwise comparison would be the same as carbon source above.

Within irrigated treatments, comparisons of MMRT parameters from different carbon sources (NPC-Rs and SOC-Rs) between sampling times showed no significant influence of either variable. In contrast to this, sampling time was a determining factor for all three MMRT parameters under dryland conditions. Pairwise comparison showed that for all three MMRT parameters, Time 5 values were significantly different

from Time 3 values. In general, these results highlight that carbon source was likely not a factor for any significant differences for all MMRT parameters; however, the treatment that soils were labelled under before sampling and sampling time were both important (Table 5.4).

When comparing Q_{10} of respiration rates from differing carbon sources and sampling times within dryland and irrigated treatments (Table 4), carbon source was a significant factor, while pairwise comparison showed that Q_{10} was mostly similar across sampling times. Overall, results suggest that temperature response (MMRT parameters) of respiration rates are influenced by treatment type and the time sampling time, while carbon source may be a significant factor in the rate of respiration and its temperature sensitivity (Q_{10}).

5.5 Discussion

Temperature response of new photosynthate carbon and soil organic carbon

There was little difference between temperature response (T_{opt} , T_{inf} , and ΔC_p^\ddagger) of respiration derived from soil organic carbon (SOC-Rs) and new photosynthate carbon (NPC-Rs) inputs within treatments (T_{inf} of 39 and 38 °C, respectively). MMRT parameters were similar to averaged irrigated values at Time 1 in Chapter 4 (T_{inf} of 41 and 39 °C of SOC and NPC, respectively).

Schipper et al. (2019) previously hypothesised that the temperature response of the biological decomposition of carbon was dependent on the balance between chemical

desorption/diffusion, which display an Arrhenius-like response, and enzyme activity which can be described by an MMRT curve (Alster et al., 2020; Schipper et al., 2019). Systems with more reliance on diffusion and sorption/desorption processes (lower substrate availability) will have higher T_{opt} and T_{inf} than those with more biologically available (not restricted by chemical or physical protection) substrate (Alster et al., 2020; Robinson et al., 2020; Schipper et al., 2019). Similar values of T_{inf} and T_{opt} for SOC-Rs and NPC-Rs suggest a similar balance of Arrhenius-like (sorption/desorption) and enzyme-activity controlling decomposition of each carbon pool (c.f. Chapter 4; Schipper et al., 2019). The observation supports previous conclusions that NPC-Rs was rapidly stabilised in soil and had a similar degradability to SOC-Rs (Chapter 4).

Comparisons of temperature response under Irrigated and Dryland treatments.

For MMRT parameters, there was no significant difference between dryland and irrigated soils; however, irrigated respiration rates were higher than the corresponding dryland rates. These results were in contrast to Schipper et al. (2019), where significant differences were found in the temperature response of respiration between irrigated (T_{opt} and T_{inf} of 70.2 and 45.5 °C respectively) and adjacent dryland soils (T_{opt} and T_{inf} of 53.7 and 32.5 °C respectively). Carmona (2020) found that although irrigation did not affect short term (< 1 year) carbon storage, it did alter the temporal allocation/cycling of carbon throughout soil physical fractions. At Time 3, immediately post-irrigation, non-rhizosphere soil under dryland had more NPC recovered than soil under irrigation (Carmona, 2020). When this recovered carbon was fractionated, most of this carbon had been transferred into the < 5 µm, mineral associated organic matter (MAOM) fraction (Carmona, 2020). Carbon associated with

the MAOM fraction is generally considered more stable and thus less available for microbial decomposition (Six et al., 2002; Wiesmeier et al., 2019). Although there was no observable difference in the temperature response of soil respiration between irrigated and dryland soil treatments in this study, only one irrigation period (three months of irrigation) had occurred. On the other hand, soils tested in Schipper et al. (2019) had been irrigated for up to 20 years. If seasonal irrigation application continued, the changes in carbon allocation observed by Carmona (2020) may, over time, lead to a similar significant difference in the temperature response of soil respiration similar to those observed in Schipper et al. (2019).

In Chapter 4, soils were labelled under either irrigated or dryland treatments, then chased under equivalent conditions. Very simply, this was to discern how new carbon inputs to soil (NPC) differed under the two moisture treatments. Overall, we showed that carbon under dryland conditions (T_{inf} of 33 and 29 °C for NPC and SOC) had the same temperature response to carbon under irrigation (T_{inf} of 39 and 41 °C for NPC and SOC). For the current study, labelling occurred under the same constant conditions, followed by a three-month moisture treatment period, representing seasonal irrigation, allowing the observation of irrigation/dryland effects on carbon cycling in soil (Carmona, 2020). A lack of difference in temperature response here, compared to that observed in Chapter 4, indicated that the overall temperature response of soil respiration was likely a result of initial stabilisation of carbon inputs to soil rather than from subsequent carbon cycling under irrigation.

Irrigation is well known to cause differences in root and shoot growth and chemical composition resulting in differences in the retention and decomposition of root-

derived carbon compared to residue/litter carbon (Córdova et al., 2018; Fulton-Smith & Cotrufo, 2019; Kong & Six, 2010). Therefore, differences in initial carbon stabilisation are expected to arise from differences in either location of carbon input (above-ground or below-ground; Fulton-Smith & Cotrufo, 2019) or carbon quality (recalcitrance or C:N ratio; Dungait et al., 2012), caused by irrigation. Chapter 4 proposed differing Q_{10} values could indicate a difference in carbon quality between irrigated and dryland treatments. However, carbon availability was a more likely cause of changes in the temperature response of respiration as root and shoot inputs were shown to have similar temperature responses despite differing Q_{10} values (Chapter 4). It was, therefore, possible that differing location of carbon input was driving the availability of carbon for decomposition in this current study.

Changes in temperature response over time, under constant conditions.

Due to the considerable variation in respiration data at Time 5, it was difficult to ascertain a continuing effect of irrigation or dryland treatments on measured parameters. However, similar to Chapter 4, pairwise comparison of times showed MMRT parameters and Q_{10} of the irrigated treatment did not change over the sampling period. Any differences in temperature response under dryland and irrigated treatments were most likely due to the lack of water availability under dryland condition, while irrigated conditions remained stable.

Temperature sensitivity

Carbon source (NPC-Rs or SOC-Rs) appeared significantly influence on temperature sensitivity, with NPC-Rs appearing to be less temperature sensitive than SOC-Rs in most cases (higher Q_{10}). This difference was comparable to observations in Chapter 4,

and again this difference in temperature sensitivity suggested that NPC was more recalcitrant than SOC to decomposition. However, this conclusion was inconsistent with the theory that older carbon is likely more recalcitrant (Conant et al., 2011). These results add further support to the observations in Chapter 4 and the suggestion that old carbon is not necessarily more chemically recalcitrant in nature but rather more stabilised in the soil (Dungait et al., 2012).

5.6 Conclusions

This study compared changes to the temperature response of soil respiration from ^{13}C enriched new carbon inputs to soil, labelled under the same moisture conditions followed by seasonal irrigation or dryland treatments. Overall, there was no significant difference between the temperature response of soil respiration from new photosynthate carbon (NPC) and soil organic carbon (SOC) within subsequent irrigated or dryland treatments. These results were similar to results reported in Chapter 4, where labelling occurring under dryland and irrigated treatment resulted in no difference in the temperature response between NPC and SOC.

When comparing the effects of subsequent irrigated and dryland treatments on the temperature response of soil respiration, no significant difference was observed between the two treatments. In contrast to this, in Chapter 4, the average temperature response (T_{inf} and T_{opt}) of irrigated and dryland treatments was significantly different. In general, similarities in temperature response between soils treated with irrigation and dryland soils seen here indicate that when differences in temperature response are seen in the field (Schipper et al., 2019), they are more likely the result of irrigation

increasing the immediate turnover/stabilisation of live plant inputs rather than subsequent carbon cycling in the soil. Focusing future carbon stabilisation research efforts on managing live root inputs during irrigation periods rather than above-ground carbon additions and later carbon cycling will be beneficial.

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Conflict of Interest

Project was funded by the New Zealand Fund for Global Partnerships in Livestock Emissions Research (SOW12-GPLER-LCR_PM).

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Chapter 6. Conclusions and Future Research

Biogeochemical cycles are important controllers of nutrient flows within and between ecosystems. Of particular importance is the global carbon cycle as it includes the cycling of carbon dioxide (CO_2), a key controller of the earth's climate, food production and soil stability (Lehmann & Kleber, 2015; Rousk & Bengtson, 2014). Soil organic carbon is the largest reservoir of actively cycling carbon. The storage of which is controlled predominantly by the balance of photosynthetic inputs to soil (plant death, root exudates) and microbial decomposition (Friedlingstein et al., 2019; Janzen, 2004; Schlesinger & Andrews, 2000). Although photosynthetic inputs are important, microbial decomposition, in particular, is sensitive to small changes in environmental factors such as temperature, moisture and pH, that control microbial growth potential of carbon supply (Conant et al., 2011; Davidson & Janssens, 2006). Additionally, anthropogenic activities such as agriculture can also impact microbial decomposition, so understanding how microbial respiration may change in response to temperature and land management is crucial for future food security and changing climate (Brusseau, 2019; Rousk & Bengtson, 2014).

The observed temperature response of microbial respiration results from two individually temperature dependant processes; 1) the indirect effect of temperature on substrate availability and 2) the direct effects on microbial metabolism (Conant et al., 2011). Although a significant amount of research does exist which investigates the temperature response of respiration of multiple ecosystems, soil types, and different management practices, varying methodologies and confusing terminology tend to limit

community consensus (Conant et al., 2011; Luo et al., 2016; Wieder et al., 2015). Robinson et al. (2017) addressed two major methodological concerns observed in the literature which could alter observed temperature sensitivity. The first concern is the use of insufficient incubation temperatures (2-6 individual temperatures), which leads to increased errors when fitting complex models to the collected data (Robinson et al., 2017). The second concern is the use of long incubation periods (7-700 days), which can allow changes to total carbon, nitrogen and microbial biomass, or thermal adaptation on microbial communities (Bradford, 2013). Robinson et al. (2017) developed a method to measure the temperature response of soil respiration a 5-hour incubation period, at 30+ discrete temperatures along a temperature gradient ($\sim 2 - 50$ °C). They demonstrated that below 20 sample temperatures points, confidence in model fits declined substantially, making it easier to fit numerous models with some justification (Robinson et al., 2017).

The most commonly used models which fit most respiration data are the Lloyd and Taylor model and the Arrhenius function. While useful models, which do fit limited data sets, these are exponential models and do not account for the decline in rate past a temperature optimum as seen in most biological processes (Alster et al., 2020; Robinson et al., 2017; Schipper et al., 2014). Macromolecular rate theory (MMRT) theory is based on the hypothesis of changing heat capacity during enzyme catalysis with changing temperature (Alster et al., 2020; Schipper et al., 2014). This model captures the decline in biological rates and includes potentially useful new parameters such as the T_{opt} (the temperature at which respiration is maximal) and T_{inf} (temperature point at which the change in respiration rate is greatest) for analysis of temperature response (Alster et al., 2020; Schipper et al., 2014).

One limitation of the methodology from Robinson et al. (2017) was that it only analyses the temperature response of respiration from total soil organic matter decomposed during incubation. However, it is widely accepted that carbon in soil is not of the same stability or complexity throughout the soil profile (Conant et al., 2011; Luo et al., 2016; Wieder et al., 2015). In general, although multiple methods of separating pools are used in the literature, it can be broadly assumed that there are at least two pools of carbon in soil. Firstly, an actively cycling, labile pool that is readily available for microbial consumption; and secondly, a stored pool which is more protected from microbial decomposition (Dungait et al., 2012; Lehmann & Kleber, 2015; Schmidt et al., 2011). These stores of potentially stabilised carbon are governed by a multitude of biological, chemical and physical processes that may react differently to temperature changes than more actively cycling soil carbon (Conant et al., 2011; Davidson & Janssens, 2006). Consistently defining and separating soil carbon into separate functional pools to understand individual temperature sensitivities is vital for accurate modelling and prediction of carbon cycling (Conant et al., 2011; Davidson & Janssens, 2006). Multiple methods of separating and defining carbon pools in the literature has resulted in inconsistent results on the temperature sensitivity of different carbon pools in soil (Conant et al., 2011; von Lützow & Kögel-Knabner, 2009; Wang et al., 2019). While there is a common consensus that labile carbon is more temperature sensitive (as assessed by Q_{10}), there is considerable variability across ecosystems, land-uses, and different methodologies (Conant et al., 2011; von Lützow & Kögel-Knabner, 2009; Wang et al., 2019).

In addition to understanding physical drivers of carbon cycling, like temperature, it is also essential to understand how different land uses, and management practices may influence the temperature response of soil respiration (Mudge et al., 2017; Whitehead

et al., 2018). In New Zealand, irrigation is a crucial management practice that may influence carbon cycling and storage within the soil. Typically, irrigation can increase above-ground biomass while potentially increasing microbial decomposition (Scott et al., 2012; Trost et al., 2013). Studies on the differences in soil organic carbon under irrigation report inconsistent findings with long-term and short term studies finding increases, decreases and no change to soil carbon under irrigation (Condrón et al., 2014; Kelliher et al., 2012; Mudge et al., 2017; Schipper et al., 2013). Carmona et al. (2020) used enriched CO₂ (¹³C-CO₂) pulse labelling to compare the mechanisms of carbon allocation under irrigation to dryland systems. They found that total new carbon inputs to soil did not increase over a three-month seasonal irrigation period. However, irrigation increased the amount of new carbon (¹³C enriched carbon) recovered in the fine particulate organic matter (POM) fraction (53–250 µm) and the clay fraction (< 5 µm) compared to dryland treatments (Carmona et al., 2020). They suggested that more new carbon in the POM and clay fractions was due to increased biological activity under irrigation, likely increasing soil aggregation and root turn-over (Carmona et al., 2020). However, whether irrigation affected soil respiration from different carbon sources was not the objective of their studies.

Uncertainty in both the inputs of carbon to the soil and, in particular, the inconsistencies in studies of soil respiration highlight the need for improved and detailed analysis of carbon cycling and stability in response to environmental changes and commonly used management practices. A lack of consistency in model choices and methodologies can significantly underestimate the degree to which the stable carbon pool will decompose under accelerated climate warming (Bradford et al., 2016; Zhou et al., 2018). Overall more information is needed to understand soil carbon cycling, particularly that which involves agricultural systems (Jackson et al., 2017).

The main objectives of this study were:

- 1) To create a new protocol, using ^{13}C enrichment methodologies, to measure the temperature response of respiration derived from two separate carbon pools in soil,
- 2) To compare the temperature response of respiration from new inputs of photosynthate carbon and soil organic carbon and determine the effects of seasonal irrigation or dryland moisture conditions on the temperature response of these two pools,
- 3) To determine whether the subsequent administration of seasonal irrigation alters the temperature response of respiration from new inputs of photosynthate carbon and soil organic carbon.

6.1. Evaluation of thesis objectives

Chapter 3: Contrasting temperature responses of soil respiration derived from soil organic matter and added plant litter.

This chapter focused on the developing of a new protocol to measure microbial respiration from two distinct carbon sources in one soil. Initial method development used ~ 0.004 g of $\delta^{13}\text{C}$ labelled ryegrass-clover mix was incorporated into ~ 3 g of natural abundance soil (Horotiu silt loam soil). These samples were incubated for 5 hours with resultant CO_2 analysed for total CO_2 and $\delta^{13}\text{C}$. $\delta^{13}\text{C}$ values were used to partition total CO_2 into respiration derived from litter carbon (Lit-Rs) and soil organic matter (SOM-Rs). Once partitioned, temperature response variables, such as MMRT

parameters (T_{opt} , T_{inf} and ΔC_p^\ddagger) and measures of temperature sensitivity (dR_s/dT and Q_{10}), could be calculated for statistical comparison.

It was found that in litter derived respiration had significantly low T_{opt} and T_{inf} (40 and 23 °C respectively) and a lower Q_{10} than SOM decomposition, which displayed an Arrhenius-like curve.

Results from this study provide direct evidence to support the hypothesis, first proposed by Schipper et al. (2019), that the observed temperature response of biological decomposition of soil carbon was a balance between enzyme activity and physical chemistry, both of which are individually temperature dependent (Figure 6.1). When substrate is limited in the soil, decomposition is rate-limited by desorption and diffusion of substrate to microbes for consumption. These processes are controlled by physical chemistry and can be described by an Arrhenius response curve (Schipper et al., 2019). When the substrate is available for consumption, the rate-limiting process for decomposition of carbon is dependent on the rate enzyme activity, which is described by an MMRT response curve, with a distinct temperature optimum (Schipper et al., 2014).

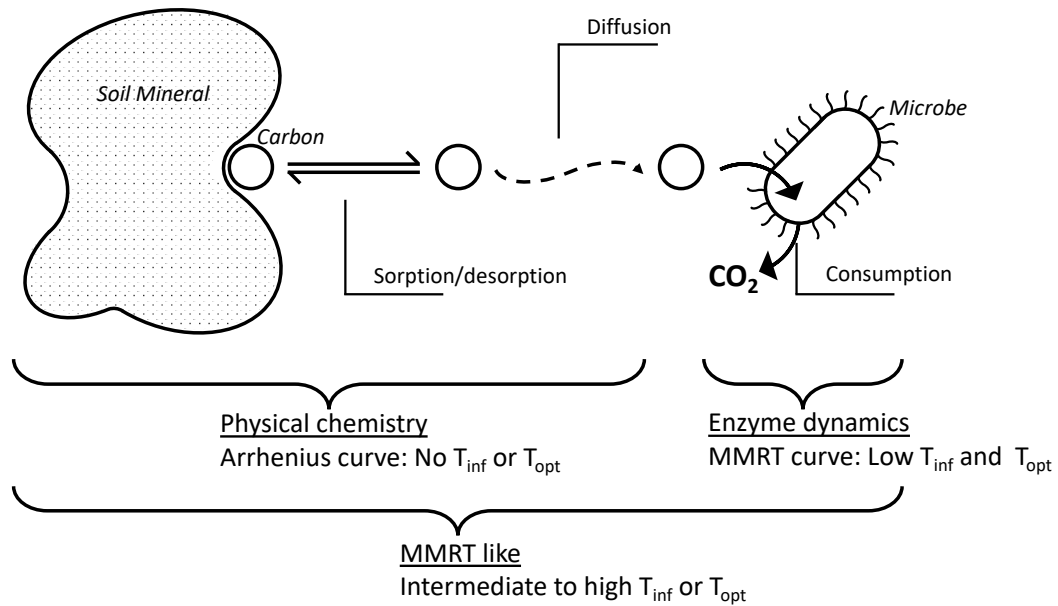


Figure 6.1: Conceptual model of the total temperature dependence of soil decomposition. Physical processes which constrain substrate consumption by microbes, such as sorption/desorption and diffusion, have an Arrhenius response to changes in temperature. Enzyme processes, on the other hand, follow an MMRT-like curve. Observed temperature dependence is the balance of these two processes depending on the substrate available for microbial consumption.

The observed mixed temperature dependence, when fitted with MMRT, can allow for assessment of the processes that predominate in a soil system and create new temperature response comparisons between different soil ecosystems (Alster et al., 2020; Schipper et al., 2019). Systems with a greater substrate availability (not restricted by chemical or physical protection) for microbial decomposition will have a more curved response and a lower T_{opt} and T_{inf} , while systems where decomposition is limited by diffusion and sorption/desorption, processes will have higher T_{opt} and T_{inf} (Alster et al., 2020; Robinson et al., 2020; Schipper et al., 2019).

This work was focused on developing a methodology for measuring two distinct carbon pool on soil and used previously labelled litter mixed with unlabelled soil.

However, there are some considerations around isotope fractionation, which need to be considered, which could theoretically lead to errors. One possible source of fractionation was the preferential selection of ^{12}C for consumption by microbes during decomposition (Brüggemann et al., 2011; Fernandez et al., 2003). Due to very high enrichment levels, this error was not considered significant; however, in samples with lower enrichment or lower respiration rates, there may be the potential to underestimate the contribution of enriched plant litter to respired CO_2 (Fernandez et al., 2003). Another source of possible fractionation is the use of syringes to withdraw gas for total CO_2 analysis on an infra-red gas analyser (IRGA). As the needle draws gas, lighter ^{12}C may get favourably pulled into the syringe, leaving a higher abundance of heavy ^{13}C behind. Similarly, when gas samples are drawn into the isotope analyser chamber and trapped for analysis, there is the same potential for more ^{12}C to be pumped into the system quicker, and thus the isotopic ratio of the trapped gas may not truly represent the original respired ratio. At this stage in the study, as the focus was mainly on method development and again, due to the high enrichment ratio of the initial litter source, this fractionation was not considered significant. In subsequent experiments, this source was eliminated after modifications to the methodology by running the entire CO_2 sample through the LGR isotope analyser without chamber trapping or gas removal. Additionally, a continuous flow baseline carrier gas (0.15 % CO_2 , 1% Ar, and 21% O_2 , in N_2) was added to improve the analysis of low CO_2 concentrations.

Chapter 4: Respiration derived from new photosynthate carbon and soil organic carbon have the same temperature response in irrigated and dryland soils.

While the previous chapter focussed on method development, this chapter primarily used the method to analyse the temperature response of soil organic carbon and new photosynthetic carbon that entered into the soil under irrigation and dryland treatments. Soils provided by Carmona (2020) had previously been labelled under dryland and irrigated treatments for three months using ryegrass/clover pasture grown under a highly enriched ^{13}C - CO_2 atmosphere (see Chapter 4 for more details). Soil samples were taken one day after labelling (Time 1) and at two other time periods (Time 3: 125 days and Time 5: 349 days after labelling) during the chase period when the same moisture contents were applied to soil cores. Samples of labelled root and shoot litter were also taken to use as mass litter inputs for a similar experiment to Chapter 3.

MMRT well described the temperature response of respiration from mass root and shoot litter inputs with a T_{inf} of about 22 and 20 °C (Dryland) and 31 and 18 °C respectively (Irrigated). Like in Chapter 3, these low values for T_{inf} were attributed to the relatively high availability of carbon for microbial consumption of the ground litter compared to soil organic carbon (SOC-Rs; Figure 6.1). Soil respiration from new photosynthetic carbon inputs (NPC-Rs), on the other hand, had an Arrhenius-like response to temperature similar to that of SOC-Rs. Similar values for T_{inf} for NPC-Rs and SOC-Rs suggested a similar balance between sorption/desorption and enzyme activity controlling the decomposition for both of these carbon pools. Unlike mass litter inputs, this similar balance indicated that either NPC entered the soil in a state that was equally consumable by microbes to that of SOC, or that within the three-

month labelling ^{13}C - CO_2 phase, new carbon was quickly stabilised in soil and subsequently reached a similar state of degradability. These results were consistent with Sokol et al. (2019), who showed that living root inputs of carbon to soil were up to 13 times more efficient at forming SOC than root or shoot litter inputs.

The effects of dryland and irrigated treatments on the temperature response of soil respiration (T_{inf} of 31 and 40 °C respectively) were similar to results from field-based measurements of the temperature response of irrigated and adjacent dryland pastures (32.5 and 45.5 °C respectively) reported by Schipper et al. (2019). They suggested that the higher T_{inf} of irrigated soil was due to a lower carbon availability than dryland soils (Figure 6.1; Schipper et al., 2019). These results were consistent with findings in Carmona et al. (2020), who found that although irrigation did not change total soil carbon, it did alter the allocation of carbon, with a significantly larger proportion being found in the mineral associated fraction ($< 5 \mu\text{m}$ MAOM fraction). It is widely accepted that carbon associated with this clay fraction is relatively stable and, therefore, less available to microbes for consumption (Six et al., 2002; Wiesmeier et al., 2019).

Pulse-labelling is used to identify pathways of carbon transfer in soil by applying an isotopic label to plants over an extended period of time (Paterson et al., 2009). The greatest limitation to pulse-chase labelling is that accurate quantification of carbon fluxes relies on the uniform spatial and biochemical distribution of the isotope label throughout the plant-soil system, which is very difficult to achieved (Paterson et al., 2009). Carbon distribution is a continuous dynamic process that can lead to the overestimation of the importance of newly incorporated carbon relative to stored carbon (Paterson et al., 2009). Additionally, exchanges between storage and transport

pools continue after the labelling period, as do carbon losses from the system. Consequently, the total labelled proportion of a flux such as respiration is uncertain and will vary due to multiple factors such as plant type and environmental condition (Paterson et al., 2009). Additionally, as mentioned above, biological processes such as photosynthesis and microbial respiration have a bias to ^{12}C , contributing to a non-uniform distribution of labelled carbon.

In this study, the isotope value for root material was considered an acceptable estimation of new, available carbon inputs to soil that occurred during the labelling period. However, as mentioned above, as labelled carbon gets redistributed throughout the profile and root ^{13}C values may become less representative of new available carbon in soil. Using a different ^{13}C value as an end member when applying the mixing model, rather than root values, was considered; however, it is expected this would not change the measured temperature response in terms of MMRT values, which were the basis of most comparisons in this study. More work is needed to understand the dynamics of this system if natural carbon inputs are to be used as a new carbon source in the future.

Chapter 5: Short-term effects of irrigated and dryland treatments on the temperature response of respiration from soil organic matter and new photosynthetic carbon.

A second experiment was conducted by Carmona (2020) using the same labelling methodology as Chapter 4. The key difference was that labelling occurred under constant spring moisture contents, after which dryland and irrigated treatments were applied for three months. Samples were taken from the day after labelling (Time 1),

after the three-month treatment phase (Time 3: 140 days after labelling ceased), and after a period of similar moisture content (Time 5: 343 days after labelling ceased).

As with Chapter 4, the temperature response of soil respiration from NPC and SOC were statistically the same. Of difference to Chapter 4 was that the temperature response of respiration rates from labelled soils, subsequently treated with dryland and irrigation moisture contents, were statistically the same (Dryland: T_{inf} of 33 and 29 °C for NPC and SOC, Irrigation: T_{inf} of 39 and 41 °C for NPC and SOC). These results indicated that the one-off application of seasonal irrigation did not affect the availability of the carbon deposited before irrigation for microbial consumption compared to dryland soils.

This study had the same methodological limitations as Chapter 4; however, as irrigated treatment occurred after labelling, the effects of these limitations would have been more pronounced by Time 3. Therefore, this work focused on changes to MMRT parameters and Q_{10} as these values are based more on the shape of the temperature response curve rather than the proportion of total flux. As sample temperature increased, any change in the isotopic composition of measured CO_2 due to a change in microbial consumption of substrate, would still be represented in the shape of the resulting curve, despite the choice of partitioning endpoints.

This research also reinforced the need for many analysis temperatures and multiple replications. Until this experiment, the data produced in this study had tight replication curves with very low scatter. In this chapter, the data variability was high, and some replicates had somewhat differently shaped temperature response curves for no

apparent methodological reasons. The high number of data points, and replicates, allowed the fitting of data, with relative confidence, despite high variability. Repeating the complex experiments was not possible due to limited available labelled soil.

6.1.1. Synopsis

Overall, the method developed was relatively robust but will need some fine-tuning in terms of mechanics to allow easier, less time-consuming analysis of ^{13}C - CO_2 . This thesis showed confidently that mass litter inputs to soil have a different temperature response to SOC (lower T_{inf}) which was related to the relative availability of substrate for decomposition. In contrast, new carbon inputs to soil through root growth/exudation did not have a significantly different temperature response to that of SOC. These two results indicated that live root additions of carbon to soil over three months were much more stable in soil than additions of litter. This work also showed that the temperature response of new plant inputs to soil under irrigation is significantly different from dryland inputs over three months. It was suggested that subsequent, increased turn-over of new carbon under irrigation led to a reduction in carbon availability for microbial consumption. Furthermore, the temperature response of new carbon inputs to soil was not changed under three months of applied irrigation. These results indicated that short-term seasonal irrigation affects carbon inputs to soil rather than subsequent carbon cycling, and thus concentrating future research on managing live root inputs might be a pathway for increasing carbon stabilisation.

6.2 Recommendations for future work

The work in this project demonstrated a new protocol for measuring the temperature dependence of two distinct carbon sources in soil. While this method was relatively successful with a discrete labelled carbon source such as ground litter, sources that come from dynamic systems, like root inputs, come with inherent difficulties in quantifying endmember isotope values, which may be hard to overcome. This work has posed several questions that need to be addressed to improve the quality of methodology and the understanding of carbon dynamics in soil systems.

6.2.1 Improving the methodology

A major problem with this methodology was that the isotope analysis portion of the method was extremely time-consuming. Currently, the automation system for the LGR isotope analyser is set up for small 3 ml exetainers. Without automation, the carrier gas input and output needles must be manually inserted into each Hungate tube. With an estimation of ~ 3 minutes per sample, three replications of 30 Hungate tubes will take over 4.5 hours to analyse manually. When including the number of ^{13}C isotope standards and CO_2 standards also needed for analysis, the total time could take well over 8 hours which is a significant burden, bearing in mind that the person running analyses must be in the room and paying close attention at all times.

Initially, in the first stages of this project, trials were completed using dry ice to create a negative pressure in an appropriate exetainer in order to draw respired CO_2 over from the incubated Hungate tube. These efforts were ultimately unsuccessful. Using a 1 mL sub-sample, injected into an exetainer, was also trialled, but in the initial

experimental stages, gas concentrations were too low for analysis, so the whole portion of respired CO₂ was required (See Chapter 3 for more information). Creating a better way of either transferring gas samples into the exetainers that fit the current automation system or adapting the automation would be ideal and allow more samples to be completed in a shorter period of time.

6.2.2 Analysing different carbon pools and soil priming

Differences in the temperature response of different C pools in soil has often been linked to the chemical complexity of the carbon substrate being decomposed or the relative availability of carbon for microbial consumption (Fierer et al., 2005). In Chapter 3, the temperature response of litter decomposition was attributed mainly to its availability compared to SOC; however, there were no investigations of its chemical complexity. Litter substrate is composed of an array of different compounds and, during degradation, it is almost impossible to tell which are being consumed. In Chapter 4, the chemical complexity of root and shoot litter was briefly mentioned when comparing the temperature response of respiration rates from mass litter inputs. Typically, roots are considered to be more chemically recalcitrant than shoot litter (Kong & Six, 2010). In this study, there was some indication of a decline in respiration rate from this more complex source; however, statistically, comparisons could not be made due to lack of data.

Simple carbon sources can be added to soil to assess the effects of chemical complexity on the temperature response of soil respiration, using a similar method to the mass litter additions in Chapter 3. During the duration of this project, Numa (2020) ran a series of experiments using multiple unlabelled carbon substrates with a range of

chemical complexity. She measured the temperature response of the different substrates by subtracting a baseline CO₂ curve (soil without carbon additions) from the resultant soil + substrate curve. Of the seven compounds studied, despite varying total respiration rates, six substrates had a very similar temperature response with T_{inf} ranging from 19 – 25 °C. Only one compound, Dextrin, followed an Arrhenius-like curve similar to that of SOC respiration (Numa, 2020).

The main limitation of this study by Numa (2020), and one that likely occurs to some degree in Chapter 3, was the probability of soil priming interfering with results. Additions of highly available carbon substrates to the soil can cause a noticeable increase in usual SOC decomposition as microbial populations flourish under energy-rich conditions. An increase in old SOC decomposition means the combined ‘soil + substrate’ curve is actually a ‘soil + substrate + priming’ curve. As soil respiration is a measured known rate, the priming response gets added to the temperature response of the new carbon substrate causing an overestimation of the amount of CO₂ resulting from the added source. By using simple ¹³C labelled substrates and the method developed in this thesis, it would be possible to isolate the temperature response of new carbon inputs using isotopic partitioning and compare different substrates with a range of chemical complexity or sorption properties. Additionally, by comparing the temperature response of partitioned SOC to that from a usual CO₂ curve, there is a potential for the temperature response of priming to be separated from the observed temperature response, something which is currently challenging.

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Appendices

Appendix 1. Additional figures from Experiment 1 (Chapter 3).

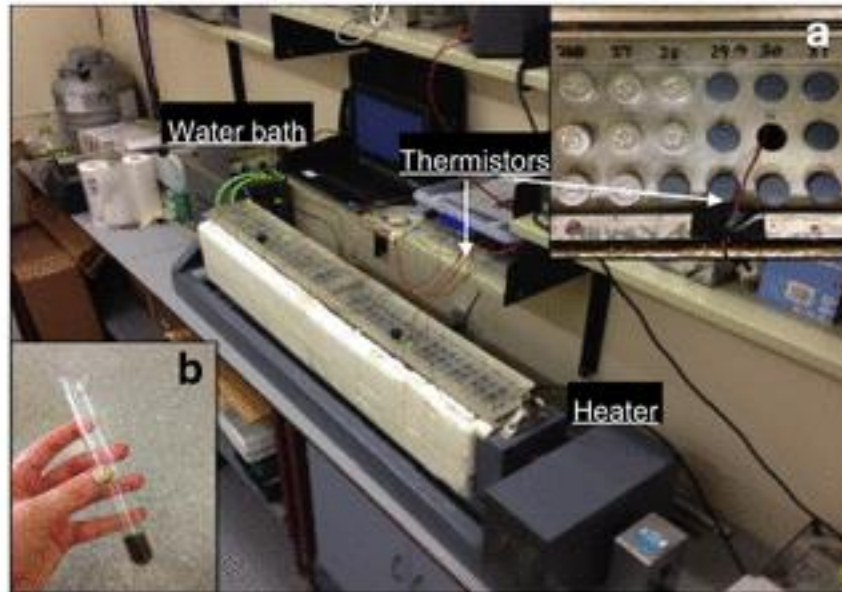


Figure A.1: Aluminium temperature block (1400 mm in length) with chilled water bath on the left end and heating element on the right. Inset a shows Hungate tubes in place, stopped on the right-hand side and crimped on the left. Inset b shows a Hungate tube with 3 g of soil.

Appendix 2. Additional data and figures from Experiment 2 (Chapter 4).

Table A.1: Calculated MMRT parameters, respiration rates, Q_{10} and dR_s/dT of from three replicates incubations of a unlabelled Limore soil. Soils were sampled 1 day (Time 1) and 125 days (Time 3) after the ‘labelling’ period.

	Treatment	T_{opt}	T_{inf}	ΔC_p^\ddagger	R_s at 10 °C	R_s at 20 °C	R_s at 30 °C	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dR_s/dT at 10 °C	dR_s/dT at 20 °C	dR_s/dT at 30 °C
		(°C)	(°C)	(J mol ⁻¹ K ⁻¹)	(µgC g ⁻¹ soil h ⁻¹)	(µgC g ⁻¹ soil h ⁻¹)	(µgC g ⁻¹ soil h ⁻¹)				(µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)
Time 1	Dryland	53	36	-2888	0.11	0.54	1.63	4.97	3.03	1.96	0.020	0.070	0.140
		67	45	-1951	0.12	0.53	1.60	4.34	3.03	2.20	0.020	0.070	0.150
		49	33	-3367	0.19	0.98	2.90	5.20	2.96	1.80	0.040	0.130	0.240
	Irrigated	44	31	-4464	0.14	0.95	3.03	6.63	3.20	1.69	0.030	0.140	0.250
		n/a*	n/a*	n/a*	0.32	1.05	2.26	3.26	2.15	1.49	0.050	0.100	0.130
		n/a*	n/a*	n/a*	0.40	0.90	1.89	2.23	2.11	2.02	0.030	0.070	0.140
Time 5	Combined	138	90	-618	0.26	0.76	1.93	2.96	2.53	2.21	0.030	0.080	0.170
		212	142	-399	0.22	0.68	1.83	3.05	2.69	2.40	0.030	0.070	0.170
		58	39	-2530	0.14	0.69	2.12	4.78	3.07	2.07	0.030	0.090	0.190

*MMRT fit produced was Arrhenius in nature, and thus these variables could not be calculated

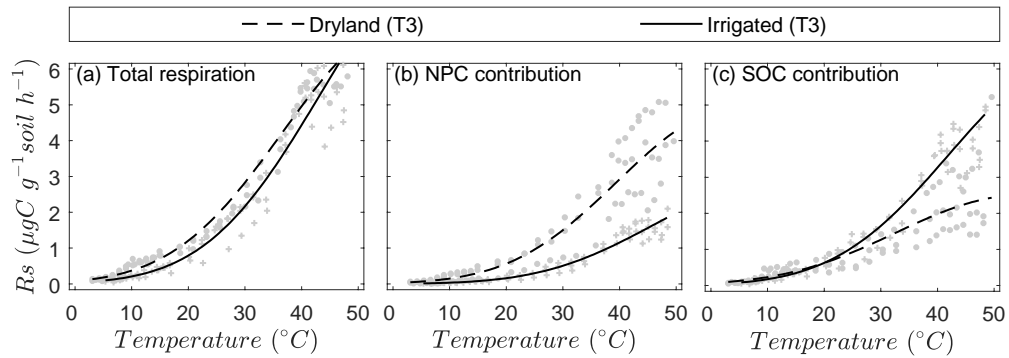


Figure A.2: Respiration rates (R_s) from a 5-hour incubation of a ^{13}C labelled Limore soil (60% MWHC), sampled 125 days (Time 3) after the end of labelling. Using a mixing model, total respiration (a) was partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c). All were fitted using the MMRT model. ^{13}C label was applied under either dryland and irrigated treatments, symbols indicate three replications of data for dryland (\bullet) and irrigated treatments ($+$), different lines represent average response for different these treatments.

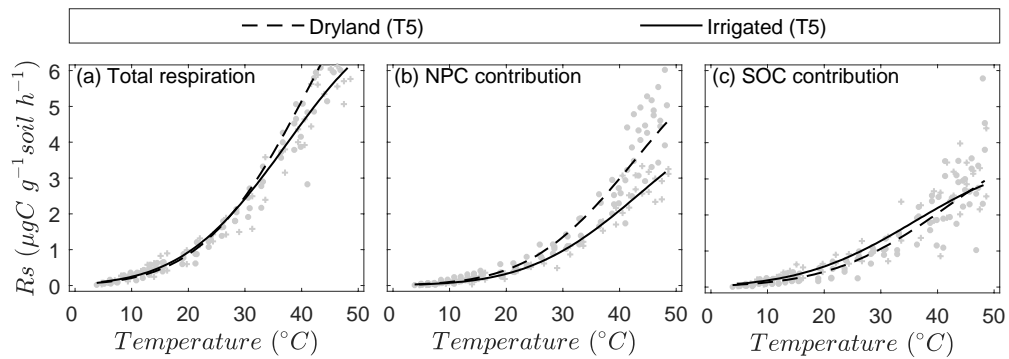


Figure A.3: Respiration rates (R_s) derived from a from a ^{13}C labelled Limore soil (60% MWHC) over a 5-hour incubation period. Soil was sampled 349 days (Time 5) after labelling had ceased. Total respiration (a) was partitioned using a mixing-model, into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c) and fitted with the MMRT model. Symbols indicate three replications of data for dryland (\bullet) and irrigated treatments ($+$), different lines represent average response for different these treatments.

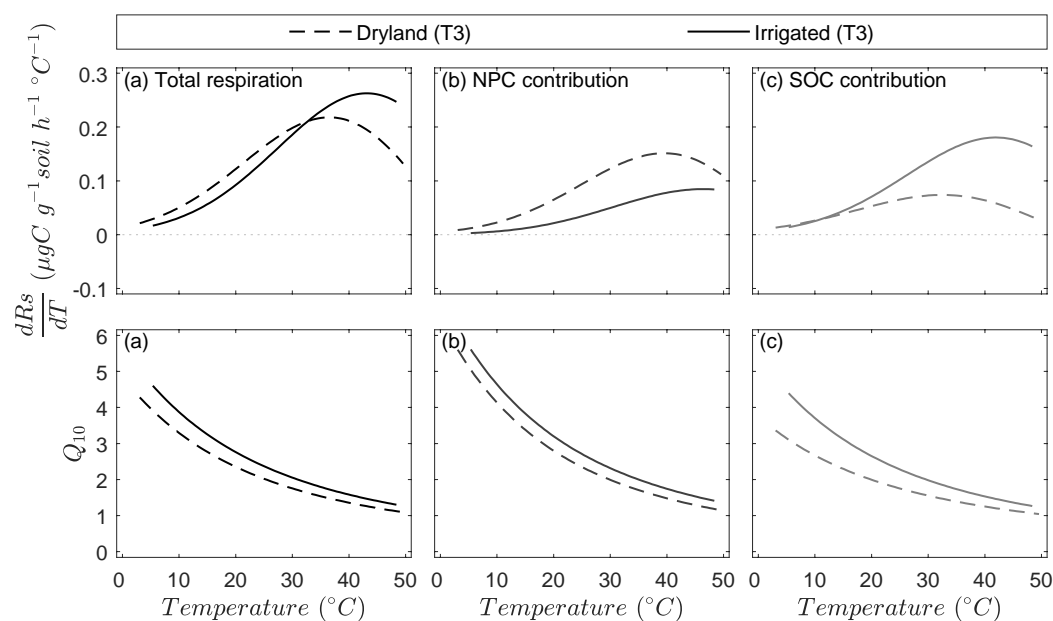


Figure A.4: Temperature sensitivity of respiration rates from ^{13}C labelled Limore soil (60% MWHC) over a 5-hour incubation. Soils were labelled under dryland or irrigated treatments and sampled 125 day after labelling (Time 3). Total respiration (a) has been partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c) and the first derivative (dR_s/dT) and Q_{10} calculated from MMRT model fits. Different lines represent average temperature sensitivity ($n = 4$) for different treatments.

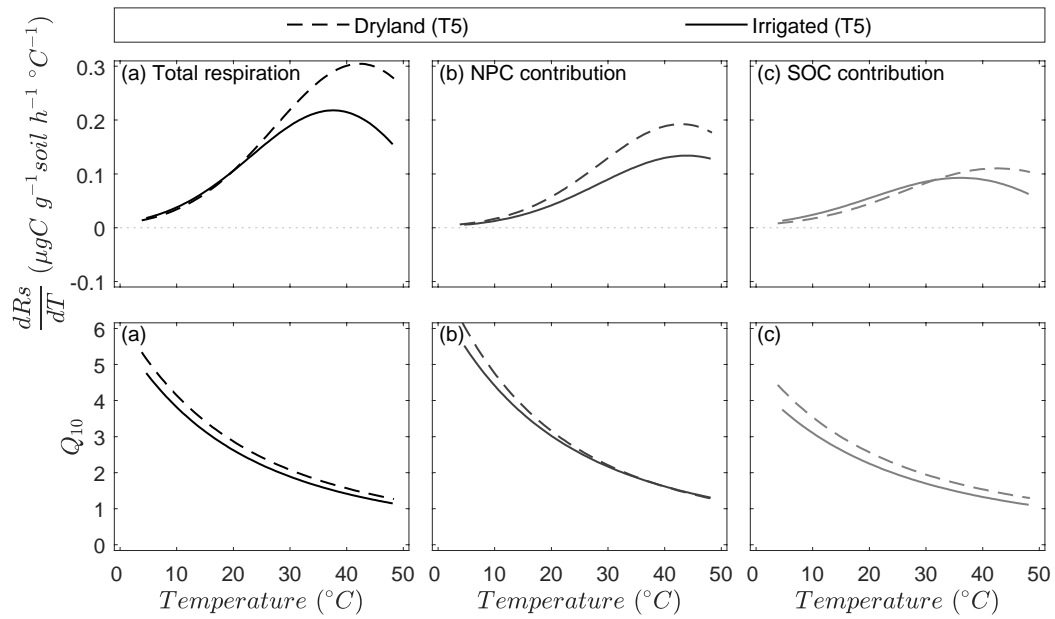


Figure A.5: Total respiration (a) from ^{13}C labelled Limore soil (60% MWHC), accumulated over an 5-hour incubation was measured and partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c). The first derivative (dR_s/dT) and Q_{10} were calculated from MMRT model fits of each respiration curve. Soils were labelled under dryland or irrigated treatments and sampled 349 days after labelling (Time 5) and different lines represent average temperature sensitivity ($n = 3$) for different treatments.

Appendix 3. Additional data and figures from Experiment 3 (Chapter 5).

Table A.2: Respiration rates (Rs) and calculated dRs/dT (mean \pm std, n= 4), derived from a ^{13}C enriched Limore soil, sampled 1 day (Time 1), 140 days (Time 3) and 343 days (Time 5) after labelling.

Treatment	C	Rs at 10 °C	Rs at 20 °C	Rs at 30 °C	dRs/dT at 10 °C	dRs/dT at 20 °C	dRs/dT at 30 °C	
	source	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	(μgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	
Time 1	NPC	0.03 ± 0.01	0.14 ± 0.04	0.46 ± 0.13	0.005 ± 0.001	0.020 ± 0.005	0.043 ± 0.012	
	SOC	0.22 ± 0.02	0.83 ± 0.04	2.22 ± 0.14	0.033 ± 0.002	0.096 ± 0.006	0.181 ± 0.013	
Time 3	Dryland	NPC	0.04 ± 0.01	0.19 ± 0.02	0.63 ± 0.08	0.007 ± 0.001	0.027 ± 0.004	0.061 ± 0.008
		SOC	0.20 ± 0.04	0.79 ± 0.12	2.15 ± 0.32	0.032 ± 0.005	0.093 ± 0.014	0.177 ± 0.025
	Irrigated	NPC	0.11 ± 0.03	0.52 ± 0.15	1.56 ± 0.44	0.020 ± 0.006	0.067 ± 0.021	0.143 ± 0.036
		SOC	0.21 ± 0.05	0.81 ± 0.10	2.11 ± 0.12	0.033 ± 0.005	0.093 ± 0.005	0.165 ± 0.007
Time 5	Dryland	NPC	0.06 ± 0.02	0.28 ± 0.09	0.92 ± 0.26	0.011 ± 0.003	0.038 ± 0.012	0.095 ± 0.021
		SOC	0.18 ± 0.02	0.79 ± 0.05	2.31 ± 0.14	0.031 ± 0.002	0.100 ± 0.005	0.205 ± 0.014
	Irrigated	NPC	0.19 ± 0.04	0.79 ± 0.29	2.39 ± 0.98	0.030 ± 0.011	0.100 ± 0.043	0.227 ± 0.098
		SOC	0.11 ± 0.07	0.39 ± 0.18	0.90 ± 0.54	0.016 ± 0.007	0.041 ± 0.019	0.057 ± 0.057

Bold values indicate the significant differences (< 0.05) between those two carbon sources, within a treatment. Respiration was sampled at the end of a 5 hour incubation period.

Table A.3: Calculated MMRT parameters, respiration rates, Q_{10} and dR_s/dT of from three replicates incubations of an unlabeled Limore soil. Soils were sampled 1 day (Time 1) and 125 days (Time 3) after the ‘labelling’ period.

	Treatment	T_{opt} (°C)	T_{inf} (°C)	ΔC_p^\ddagger (J mol ⁻¹ K ⁻¹)	R_s at 10 °C (µgC g ⁻¹ soil h ⁻¹)	R_s at 20 °C (µgC g ⁻¹ soil h ⁻¹)	R_s at 30 °C (µgC g ⁻¹ soil h ⁻¹)	Q_{10} at 10 °C	Q_{10} at 20 °C	Q_{10} at 30 °C	dR_s/dT at 10 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	dR_s/dT at 20 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)	dR_s/dT at 30 °C (µgC g ⁻¹ soil h ⁻¹ °C ⁻¹)
Time 1	Spring	58	39	-2486	0.20	0.95	2.88	4.66	3.01	2.05	0.040	0.130	0.260
		45	24	-1950	0.17	0.39	0.67	2.34	1.70	1.29	0.020	0.030	0.030
		92	61	-1194	0.22	0.84	2.45	3.73	2.92	2.34	0.030	0.100	0.230
Time 5	Dryland	237	162	-379	0.18	0.61	1.76	3.30	2.90	2.59	0.020	0.070	0.180
		96	64	-1136	0.27	1.00	2.93	3.73	2.94	2.38	0.040	0.120	0.280
	Irrigated	71	47	-1712	0.25	0.98	2.85	4.00	2.90	2.18	0.040	0.120	0.260
		58	39	-2376	0.27	1.22	3.55	4.44	2.92	2.02	0.050	0.150	0.310

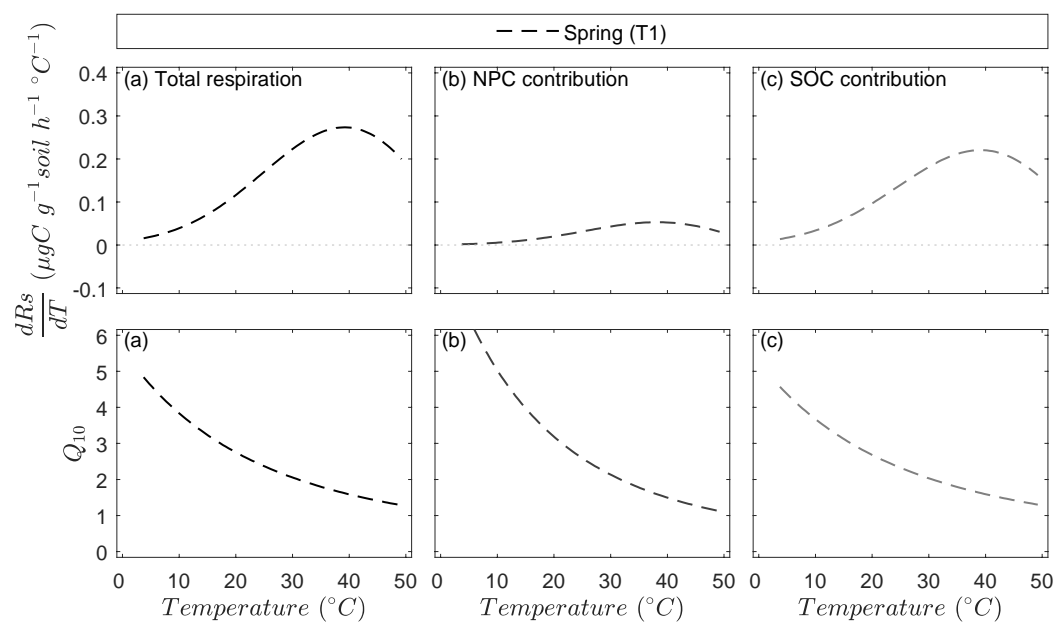


Figure A.6: First derivative (dR_s/dT) and Q_{10} calculated from MMRT model fits of respiration rates from a ^{13}C labelled Limore soil (60% MWHC), incubated for 5-hours. Labelling occurred under the same spring moisture conditions and was sampled 1 day after labelling (Time 1). Total respiration (a) was partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c).

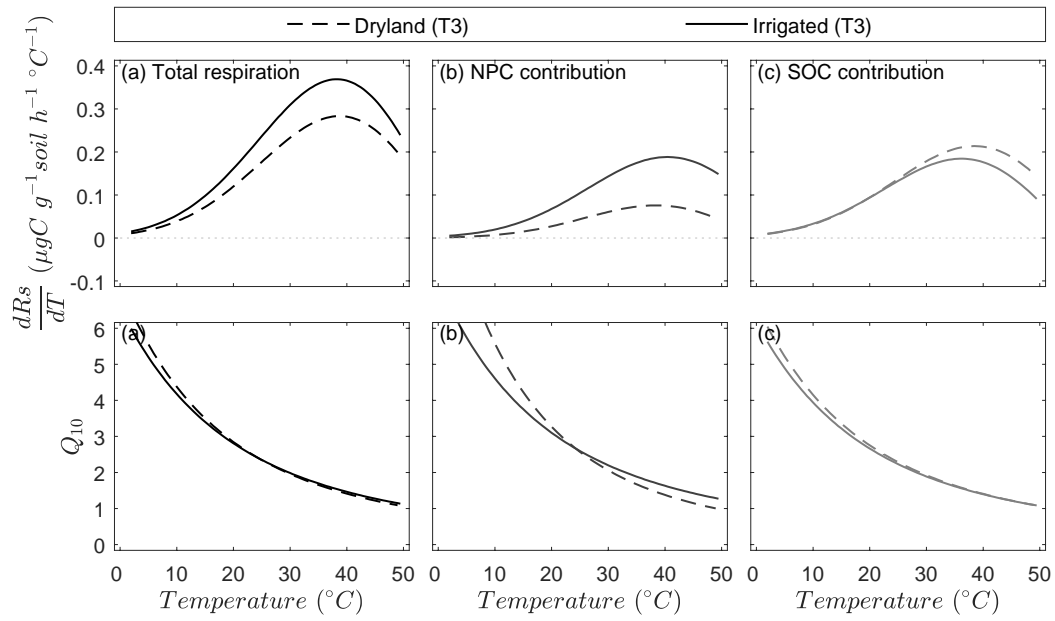


Figure A.7: Temperature sensitivity of respiration from a 5-hour incubation of ^{13}C labelled Limore soil (60% MWHC). Labelling occurred under the same spring moisture conditions followed by a three-month period of irrigation and dryland treatments. Total respiration (a) was partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c). First derivative (dR_s/dT) and Q_{10} were calculated from MMRT model fits of respiration rates from soil sampled 140 days after labelling (Time 3) and different lines represent average temperature sensitivity ($n = 3$) for different treatments.

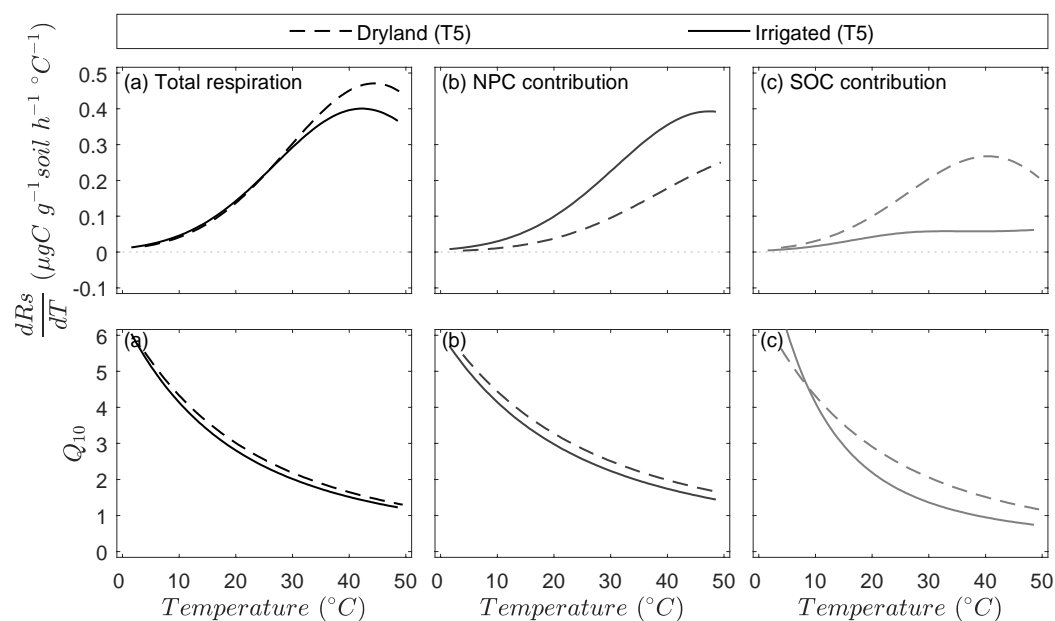


Figure A.8: Total respiration (a) T from a 5-hour incubation of ^{13}C labelled Limore soil (60% MWHC) partitioned into respiration from new photosynthate carbon (b) and bulk soil organic carbon (c). Soils were labelled under spring conditions followed by a three-month period of irrigation or dryland treatments and soil were sampled 349 days after labelling (Time 5). Resulting respiration curves were fit with MMRT and the first derivative (dR_s/dT) and Q_{10} were calculated. Different lines represent average temperature sensitivity ($n = 3$) for the two different treatments.



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In the submitted thesis, Chapter 3 has been published in the journal Biogeochemistry. The published title "Contrasting temperature responses of soil respiration derived from soil organic matter and added plant litter", is the same as that of the chapter in this thesis.

Nature of contribution
by PhD candidate

Collected, incubated and analysed soil samples including all data analysis and figures in this work.
Wrote thesis chapter and manuscript.

Extent of contribution
by PhD candidate (%)

90%

CO-AUTHORS

Name	Nature of Contribution
Louis Schipper	Project conception; collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions.
Shaun Barker	Collaboration on experimentation data analysis and interpretation; provided comments on manuscript/chapter versions.
Vickery Arcus	Collaboration on data analysis and interpretation; provided comments on manuscript/chapter versions.
Sam McNally	Provided material for experimentation, provided comments on manuscript/chapter versions.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Louis Schipper		3/8/20
Shaun Barker		18/08/20
Vickery Arcus		07/09/20
Sam McNally		1/9/20

Co-Authorship Form

Postgraduate Studies Office
Student and Academic Services Division
Wahanga Ratonga Matouranga Akonga
The University of Waikato
Private Bag 3105
Hamilton 3240, New Zealand
Phone +64 7 838 4439
Website: <http://www.waikato.ac.nz/sasdl/postgraduate/>

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In the submitted thesis, Chapter 4 represents the second experimental piece of work titled "Respiration derived from new photosynthate carbon and soil organic carbon have the same temperature response in irrigated and dryland soils." Currently, this work remains unpublished.

Nature of contribution by PhD candidate	Collected, incubated and analysed soil samples including all data analysis and figures in this work. Wrote thesis chapter and manuscript.
Extent of contribution by PhD candidate (%)	90%

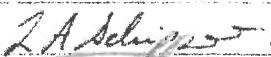



CO-AUTHORS

Name	Nature of Contribution
Louis Schipper	Project conception; collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions.
Carmen Medina	Provided material/data for experimentation; collaboration on data interpretation, provided comments on manuscript/chapter versions.
Sam McNally	Collaboration on data analysis and interpretation; provided comments on manuscript/chapter versions.
Mike Beare	Collaboration on data analysis and interpretation; provided comments on manuscript/chapter versions.

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and

Name	Signature	Date
Louis Schipper		11/8/20
Carmen Medina		17/8/20
Sam McNally		1/9/20
Mike Beare		7/9/20

Co-Authorship Form

Postgraduate Studies Office
Student and Academic Services Division
Wahanga Refonga Māteauranga Akonga
The University of Waikato
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In the submitted thesis, Chapter 5 represents the second experimental piece of work titled "Short-term effects of irrigated and dryland treatments on the temperature response of respiration from soil organic carbon and new photosynthate carbon" Currently, this work remains unpublished.

Nature of contribution
by PhD candidate

Collected, incubated and analysed soil samples including all data analysis and figures in this work.
Wrote thesis chapter and manuscript.

Extent of contribution
by PhD candidate (%)

90%





CO-AUTHORS

Name	Nature of Contribution
Louis Schipper	Project conception; collaboration on data analysis, interpretation and visualisation; provided substantial comments and editing on manuscript/chapter versions.
Carmen Medina	Provided material/data for experimentation; collaboration on data interpretation, provided comments on manuscript/chapter versions.
Sam McNally	Collaboration on data analysis and interpretation; provided comments on manuscript/chapter versions.
Mike Beare	Collaboration on data analysis and interpretation; provided comments on manuscript/chapter versions.

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